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THYMIC TUMORS CHARACTERIZED BY LYMPHANGIOENDOTHELIAL STRUCTURE *

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The thymus is still on the frontiers of investigation in biology. Neither the function nor even the structure of the normal organ is as yet completely understood.

The Hassall corpuscles are generally accepted as consisting of squamous epithelium. There is, however, no agreement concerning cells of other types. The small, spherical cells with scanty cytoplasm and dense nuclei are sometimes called thymocytes, implying that they may be different from lymphocytes. This, however, is a presumption devoid of proof. Indeed, studies with cytotoxic sera by Pappenheimer[1] have shown that both types respond similarly. There are also certain scattered, larger, paler cells regarded by some as elements of the connective tissue, while others consider them to be epithelial cells of the original thymic anlage scattered by the intrusion of masses of lymphocytes. To add to the confusion, the proponents of both points of view refer to these cells as "reticulum."[2]

Even less well understood than the normal structure of the organ is the histogenesis of tumors arising from, or in the position of, the thymus. The proximity of the bronchi and the appearance in the anterior mediastinum of teratomas, whose capacity for mimicry is well known, often render difficult a decision as to the primary source of a tumor in this region. The bronchogenic tumors are especially troublesome, since they are now of very frequent occurrence, and possess a tendency to anaplastic structure, especially when arising in the most proximal bronchi. Moreover, a non-obstructing and consequently relatively silent bronchogenic carcinoma may present itself as a predomi-

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nantly mediastinal lesion. These difficulties stand in the way of ready acceptance, as of thymic origin, of most of the variegated groups delineated by Margolis,[3] Lowenhaupt,[4] and Symmers.[5]

Most acceptable as primary thymomas are the well encapsulated, slowly growing tumors such as have been described by Seybold, McDonald, Clagett, and Good.[6] In these instances there is no evidence of involvement of the lymphoid tissue generally and extension, when it occurs, is not by way of blood vessels or lymphatics, but merely by continuous invasion of tissue, or rarely upon the surface of the pleura. The tumors consist of cells of two types: The first is the lymphocyte (thymocyte?); the second has been considered epithelium. In the present material, comprising three tumors whose general characteristics are similar to those in Seybold's series, lymphocytes again were present. Evidence will be presented, however, suggesting that cells of the second type are not of epithelial, but of lymphangioendothelial origin. The microscopic structure of the tumor in the first patient will be described in some detail as a basis of comparison with the other lesions, which differ only in minor, or quantitative, detail.

Report of Cases

Case 1

Three years prior to admission, the patient, a 71-year-old woman, developed a persistent cough yielding a small quantity of yellow, mucoid sputum. Six weeks before admission a shadow was seen upon roentgenographic examination of the chest and she was referred to New Haven Hospital. There was nothing of note in her past history.

She appeared well nourished, but coughed during the examination. The temperature was 98° F.; pulse, 88; respirations, 16; blood pressure, 150/100 mm. of Hg. Only the findings of note are given. In the left parasternal region below the clavicle, voice sounds, breath sounds, and tactile fremitus were diminished. There were a few crackling râles at the base of the left lung, and a blowing systolic murmur was heard in the precordium, but there were no thrills and the cardiac rhythm was regular.

Roentgenographic studies (Figs. 1 and 2) demonstrated a mass approximately 10 cm. in greatest diameter in the anterior mediastinum and extending to the left of the midline. There were no intrinsic pulsations, nor was there evidence of calcification. Upon bronchoscopy, the angle at the carina was more obtuse than usual, and the left main bronchus appeared to be compressed by an extrinsic mass impinging upon its medial and posterior aspects. There was hyperemia of the mucous membranes of the bronchi in the left lower lobe and the position of these bronchi was somewhat distorted. The preoperative diagnosis was probable dermoid tumor.

A left thoracotomy was performed on December 29, 1949, exposing a large, lobulated mass attached to the left side of the pericardium and mediastinum. The mass was removed without difficulty since it appeared embedded within its own capsule of connective tissue. During the first 3 days after the operation there was a temperature elevation to 100° F. associated with râles in both lungs. Thereafter, following bronchial aspiration, the temperature approached normal levels, the wound healed well, and the patient was due to be discharged on the eighth day after operation. On that day, however, she died suddenly. Permission for necropsy was not obtained.

Gross Description

The specimen removed at operation was ovoid, measured 8 by 9 by 6.5 cm., and weighed 275 gm. It was covered, for the most part, by a thin, glistening membrane whose appearance suggested serosa. On one aspect there was merely a rough capsule of connective tissue. There were small protrusions from the surface which were likewise encapsulated. Upon incision, the substance of the mass was firm. It was traversed by radiating trabeculae of firm, white material that had the appearance of connective tissue (Fig. 3). Demarcated by these trabeculae were bulging nodules of a somewhat softer, pale pink, and more translucent tissue.

Microscopic Findings

The two main components of the tumor are illustrated within the same field in Figure 4. The first part, seen above such a trabecula of connective tissue as has been described grossly, consisted of spindle-shaped or flattened cells in masses arranged about lumina. The second component consisted largely of cells indistinguishable from lymphocytes, although close inspection would reveal even here an inconspicuous admixture of spindle-shaped cells of the first type; elsewhere the admixture was more obvious (Figs. 10 to 12).

Where masses of cells of the first type abutted upon a septum of connective tissue or upon the capsule, an elongated channel remained, lined throughout by flattened elements of endothelial type (Figs. 4 to 6). This suggested that a lumen had been expanded by eccentric proliferation, in intracanalicular fashion, of a mass of cells which was itself subdivided by subsidiary lumina in "swiss cheese" pattern. This is illustrated on a smaller scale in Figure 7 where remnants of connective tissue similarly outline a much smaller cell mass. It will be noted that a part or all of each lumen is lined by flattened cells with the characteristics of endothelium. As the latter becomes continuous with the more solid cell masses they become, by gradual transition, more plump, and their nuclei are less elongated. Nucleoli are minute and the chromatin is very close meshed and finely reticular. The nuclear membrane is delicate (Fig. 7).

Where cell masses of the first type impinged upon connective tissue they might infiltrate in tongue-like, solid groups or as minute channels lined by a single layer of flattened cells. There the appearance was most highly suggestive of lymphangioma (Fig. 8). Elsewhere, near the obviously tumorous tissue, there were large, definitely lymphatic channels likewise lined by endothelium and with clearly identifiable muscle cells within their walls (Fig. 5).

Where the cells were most compactly arranged, they often were whorled, and these whorls might occur within the stroma of lymphocytes. Such an arrangement of apparently similar cells suggested to Seybold and his co-workers[6] "immature Hassall's corpuscles." In our experience, however, none of these has shown any tendency to keratinization or calcification.

Groups of these cells of the first type appeared within lymphoid tissue, in some places as prominent islands (Fig. 12), but elsewhere in less conspicuous groups, whorled, or ramifying into fascicles of spindle cells that lost themselves among lymphocytes. There was, however, a persistent tendency to the formation of small lumina where the cells occurred in masses.

Case 2

The patient was a mechanical engineer, 63 years old. Two months before admission to the hospital, routine fluoroscopy had revealed a mass in the mediastinum. He had had no significant complaints previously, but during the 6 weeks before admission he had noted some pounding of the heart when he lay upon the left side, and had a short episode of fever and a productive cough that cleared completely in a few days on penicillin therapy.

Twenty-one years previously a cardiac murmur had been noted during an insurance examination. The patient had had no dyspnea, edema, or other symptoms of cardiac disease.

Upon admission his temperature was 97° F.; pulse, 64; blood pressure, 160/95 mm. of Hg. He seemed in good health. Pertinent findings upon the physical examination were as follows: There was an area where breath and voice sounds were diminished and where dullness and fine râles were perceptible immediately to the left of the sternum, extending laterally for 3 cm. and merging into the zone of the cardiac dullness below. The heart was not demonstrably enlarged. A mid-diastolic murmur was best heard over the lower precordium. Laboratory data were non-contributory. Roentgenographic studies, including kymography, demonstrated a rounded density (Figs. 14 and 15) in the anterior mediastinum, estimated to measure 7.5 by 9.5 cm. It was visible just above the cardiac silhouette from which it was demonstrably detached. Again, neither intrinsic pulsation nor calcification was noticed. Preoperative diagnoses were dermoid tumor of mediastinum, probable interventricular septal defect (asymptomatic).

On April 19, 1948, a thoracotomy was performed through the fourth interspace anteriorly on the left side. A large, solid tumor was excised from the fibrous tissue which bound it to the pericardium, mediastinal pleura, left superior pulmonary artery and vein, and lingula. There was, however, no evidence of invasion of these structures. A cardiac thrill was palpable with the heart exposed. The postoperative course was uneventful and the patient was discharged on the tenth day. Follow-up examinations up to 2½ years later showed nothing remarkable except slight exertional dyspnea. The roentgenograms have been negative.

Gross Description

The specimen removed surgically was an ovoid mass covered by a thin capsule that was traversed by numerous thin-walled vessels (Fig. 16). Several secondary hemispheric nodules were present on the sur-

face. All cut surfaces revealed a pale, translucent, gray substance, streaked in some places by extravasated blood and, near the center, by masses of bright yellow, opaque substance. There was no evidence of calcification nor was a cystic structure discernible grossly. The tumor was much softer than that in case 1 and there were no dense trabeculae of connective tissue.

Microscopic Findings

In this tumor spindle-shaped cells and lymphocytic elements occurred in approximately equal proportions. The former were in fascicles, in large part permeated by small channels, some of which contained finely granular, acidophilic material (Figs. 18 and 19). The spindle cells became concentrically arranged about these channels and indeed appeared to line them. For the most part these spaces were small, but larger ones were found in a few places (Fig. 21). A sclerosing process appeared to be effecting the obliteration of the small vessels in certain portions of the tumor (Fig. 20). The lymphocytes were more concentrated in some fields than in others and the spindle cells became lost among them as in case 1. Only in a few places were large, compound, finely cystic structures formed (Fig. 22), as in the first specimen. Again, they abutted upon sheets of densely arranged connective tissue. Here the cells were plump but appeared to be merely transformed spindle cells. In general, argyrophilic fibers were scanty, within both the lymphoid and spindle cell components, and were most abundant in the few places where fibrosis was in progress (Fig. 23).

Case 3

A Negro high school girl, 17 years old, was admitted to the thoracic surgical service 8 months after discovery of what was thought to be an abnormal cardiac shadow as the result of a routine survey film. She had been quite well and indeed very active in athletics. There were no cardiac or respiratory symptoms. She had had each year only one or two brief episodes of upper respiratory infection with tracheobronchitis.

Upon physical examination she appeared well developed and well nourished. Her blood pressure was 110/72 mm. of Hg.; pulse, 60; temperature, 98.6° F.; respirations, 16. The lung fields were clear to percussion and auscultation. The cardiac rhythm was regular and a slight systolic murmur could be heard only after exercise. There was nothing else of note.

Roentgenographic examination of the thorax revealed a smooth, rounded, homogeneous density with sharply defined margins closely applied to the right cardiac border and extending into the right anterior mediastinum (Figs. 24 and 25). Roentgenkymography and fluoroscopy demonstrated transmitted pulsations. With the patient on her right side, the mass was seen to swing downwards away from the shadow of the heart (Fig. 26). The laboratory data were non-contributory. The preoperative diagnosis was either dermoid cyst or thymoma. A thoracotomy was performed through the right 5th interspace. This exposed a large, partially cystic, tumor mass in the anterior mediastinum just in front of the phrenic nerve, which extended a long, tongue-like projection upward toward the neck. The superior vena

cava was just lateral and posterior to the mass, and apparently normal thymic tissue lay anteriorly and medially. The pedicle was ligated and divided proximally to the main body of the mass. The postoperative course was uneventful and the patient was discharged on the eighth day.

Gross Description

The resiliently soft mass was well encapsulated. The long projection that extended upwards into the superior mediastinum was covered by a reflection of the capsule and had a more firmly resilient consistency than the remainder of the tumor. The mass weighed 69 gm. and had major dimensions of 10 by 6 by 4 cm. Most of it consisted of a cystic structure containing a clear, thinly mucoid, pale yellow fluid. At least three-fifths of the space within the cyst was occupied by very soft masses, encephaloid in their pale yellow-gray color and translucency, that projected from a firmer wall of tissue averaging 1.5 mm. in thickness (Figs. 27 and 28). Especially near the lower pole the tissue again was resiliently firm and rendered spongy by the presence of innumerable minute spaces that were filled with clear fluid.

Microscopic Findings

Histologically, this tumor consisted predominantly of lymphoid substance. This was the only one of the three in which Hassall's corpuscles were found (Fig. 29). Some had become greatly enlarged and partially calcified (Fig. 30). Especially near the lower pole, there were numerous large, ramifying spaces lined by flattened cells. Some of these contained a homogeneous acidophilic material. They again had the structure of lymphatics. About some there had been hyalinization of connective tissue (Fig. 31). Muscle cells were not clearly defined in the walls of these spaces. In the septa there were scattered lymphocytes. In portions of the lymphoid tissue there occurred masses of spindle-shaped elements, similar to those in the other tumors of this series (Fig. 33). These cells tended to arrange themselves about minute spaces. Definite transitions to the large channels were not discerned, however. The argyrophilic reticulum again was scanty (Fig. 34).

Discussion

One may question the basis on which these tumors are considered to be of thymic origin. Their position in the mediastinum, their well encapsulated state, and their general similarity to the group described by Seybold and co-workers[6] are compatible with such an origin. The presence of Hassall's corpuscles in case 3 is conclusive. These structures, however, were not demonstrated in the tumors of cases 1 and 2 although the latter differed from one another only in the relative pro-

portions of the different types of tissue of which they were composed.

The most significant evidence for the lymphangioendothelial nature of a principal component of these tumors is the presence of transitional forms, from lymphatics containing muscle fibers in their walls, to spaces lined by proliferated endothelial cells, to solid masses of the latter, and finally to spindle-celled elements that lose themselves among the lymphoid cells of the stroma. These transitions are best demonstrated in the first tumor. Certain features of the few available adequate illustrations of thymic tumors that have been published indicate that the three tumors in the present series are not unique. Thus, in the article by Seybold, McDonald, Clagett, and Good,[6] parts of Figures 7 and 8 could be taken as illustrative of lymphangioendothelioma, rather than of gland formation on the part of epithelium, and this has been admitted as a possibility in a personal communication from Dr. McDonald. Seidel[7] reported upon a microcystic mediastinal tumor in a 2½-year-old child under the diagnosis of lymphangioma of the thymus. Other mediastinal tumors of this type have been described, but whether they were associated with the thymus is not clear. The most suggestive was reported by Sanes, Macmanus, and Scatchard[8] and a similar instance by Michaelis.[9] The tumor described by Skinner and Hobbs[10] was of very great size, involving much of the right side of the chest and extending to the diaphragm. We have seen cystic tumors attached to the fatty tissue in the region of the foramen of Morgagni of the diaphragm that could be confused with "pericardial cysts" but which were clearly lymphangiomatous. The rare cystic lymphangiomas of the neck may have large intrathoracic extensions.[11-13]

The presence of lymphangioendothelium in a tumor of thymic origin may be questioned, since it has been stated that the medulla of this organ, like the spleen, contains few if any lymphatics.[2] Lymphatics, however, exist within the cortex and, in abundance, within the stroma. This is to be expected since the thymus lies astride the dense lymphatic networks of the mediastinum. That the proliferative process should involve both the lymphocytes and the lymphangioendothelium of this organ is a remarkable and unexplained phenomenon.

There was no suggestion of myasthenia gravis in any of the three patients in the present series. In Seybold's series[6] three-fourths of the patients with thymic tumors suffered from myasthenia. This incidence of myasthenia gravis is probably weighted by an interest in treatment of the disease at the Mayo Clinic. The relationship between the tumors and myasthenia is not clear, since only 15 to 20 per cent of patients with myasthenia have a demonstrable lesion in the thymus.

Summary and Conclusions

In each of three well encapsulated tumors of the thymus removed surgically, within a stroma rich in lymphocytes, there were apparently lymphatic spaces whose endothelial cells had proliferated focally with reduction of the lumen. Elsewhere, these cells infiltrated the stroma wherein they gradually assumed a more elongated shape and became scattered among the lymphocytes. These observations suggest that the possibility of a lymphangioendothelial component should be considered in the study and classification of tumors of the thymic region.

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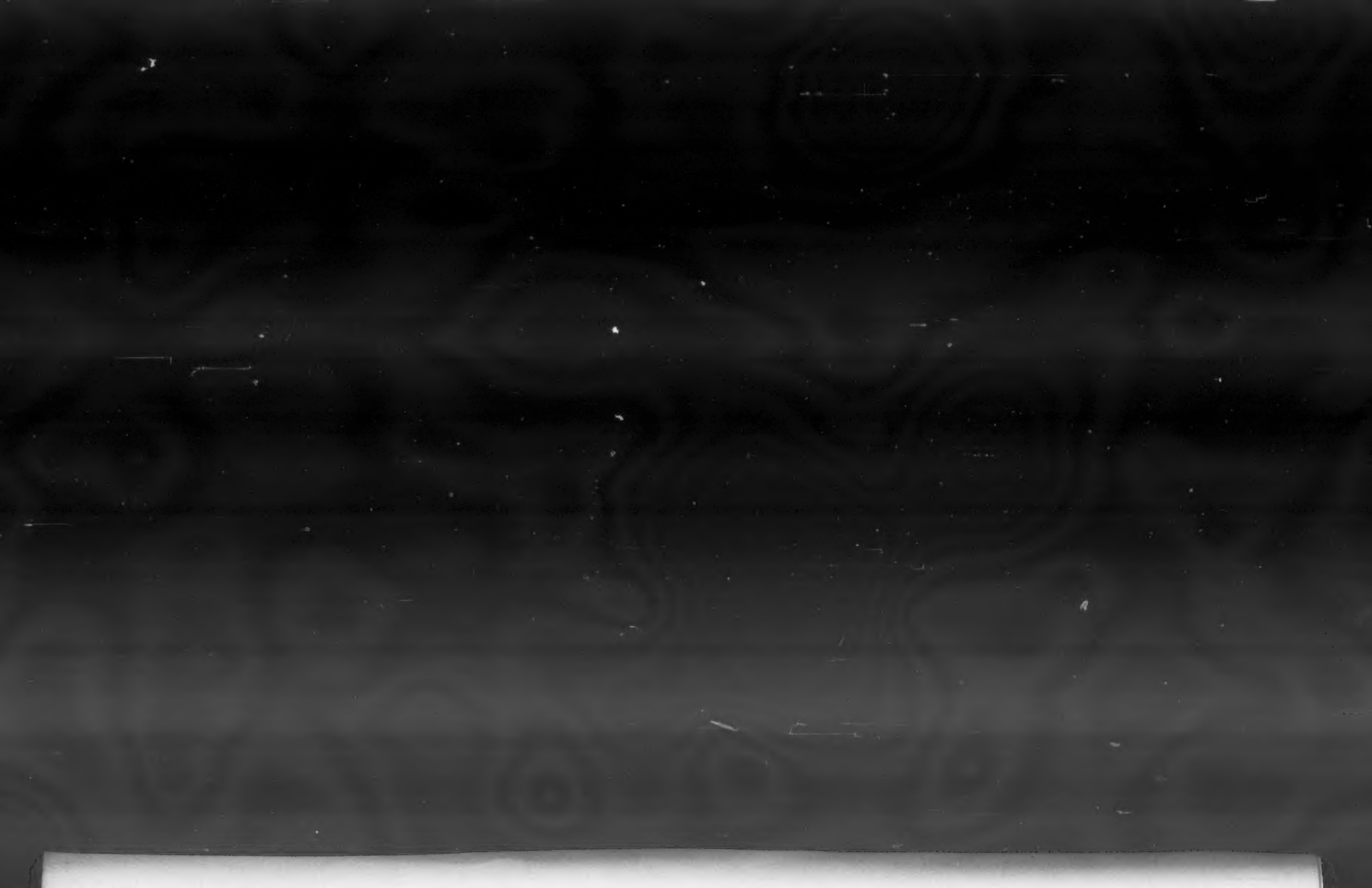
DESCRIPTION OF PLATES

Plate 45

Fig. 1. Case 1. Roentgenogram. Lateral view. Anterior position of ovoid mediastinal mass.

Fig. 2. Case 1. Posterior-anterior roentgenogram, showing protrusion of tumor to the left from the anterior mediastinum.

Fig. 3. Case 1. Transected gross specimen showing radiating fibrous trabeculae among which are bulging masses of a more translucent tissue.



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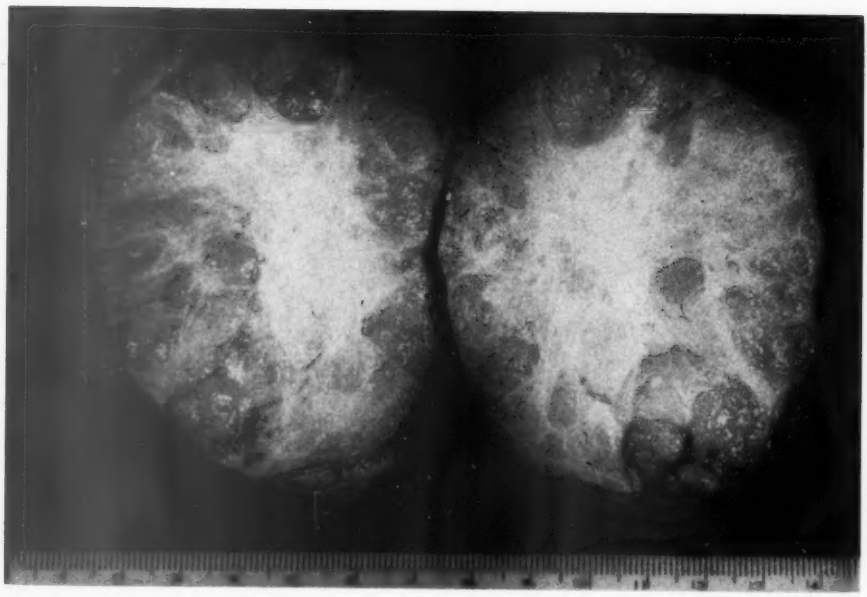

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Hubbell and Liebow Lymphangioendothelial Thymic Tumors

PLATE 46

FIG. 4. Case 1. The two types of tissue are shown respectively above and below a connective tissue trabecula. Flattened cells are seen above the trabecula toward the right. Continuous with these cells at the left are masses of somewhat plumper cells separating smaller spaces in a "swiss cheese" pattern. Below the trabecula the tissue is composed largely of lymphocytes, but here, too, there are spaces and spindle-shaped cells.

FIG. 5. Case 1. A lymphatic with smooth muscle in its wall is seen at the center of the bottom of the photomicrograph. To the right a similar channel is lined in part by piled-up cells as in Figure 4.

FIG. 6. Case 1. A section of the tumor near the capsule. Bulging into an elongated endothelium-lined space near the capsule is a compound mass of proliferated endothelial cells, itself penetrated by smaller channels. The subcapsular space and many of the subsidiary channels contain a homogeneous acidophilic substance.

FIG. 7. Case 1. Detail of Figure 5. In the upper central portion, outlined by remnants of connective tissue, is a large channel with a lumen made eccentric by a cushion of proliferated cells that elsewhere are arranged in a single layer of flat endothelium. Other channels are seen in the same field. The higher magnification demonstrates the delicate, very finely reticular nuclear structure of these cells, and the minute, often multiple, nucleoli. The cytoplasm is in an elongated, neutrophilic, homogeneous mass.

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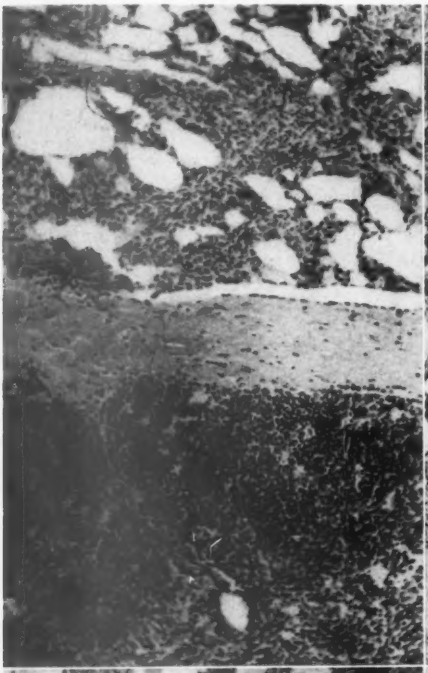

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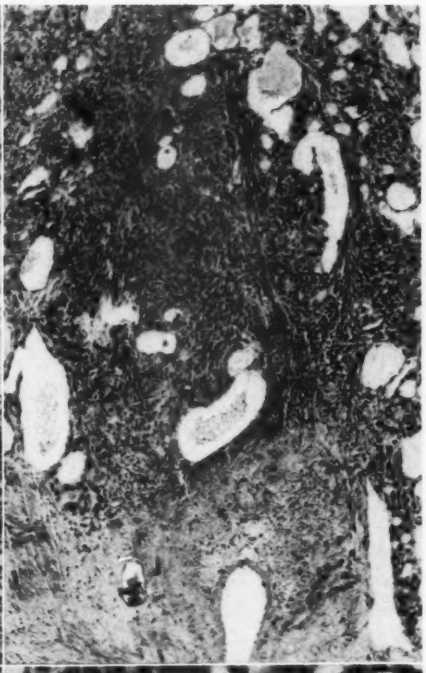

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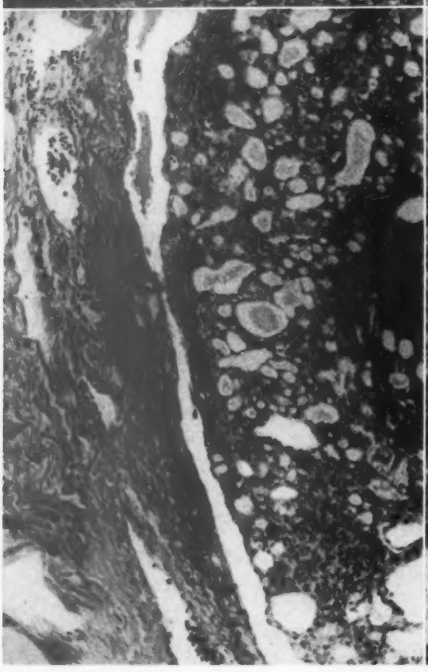

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Hubbell and Liebow Lymphangioendothelial Thymic Tumors

PLATE 47

FIG. 8. Case 1. Within a dense fibrous stroma many channels of varying size are seen to be lined by a single layer of flattened cells.

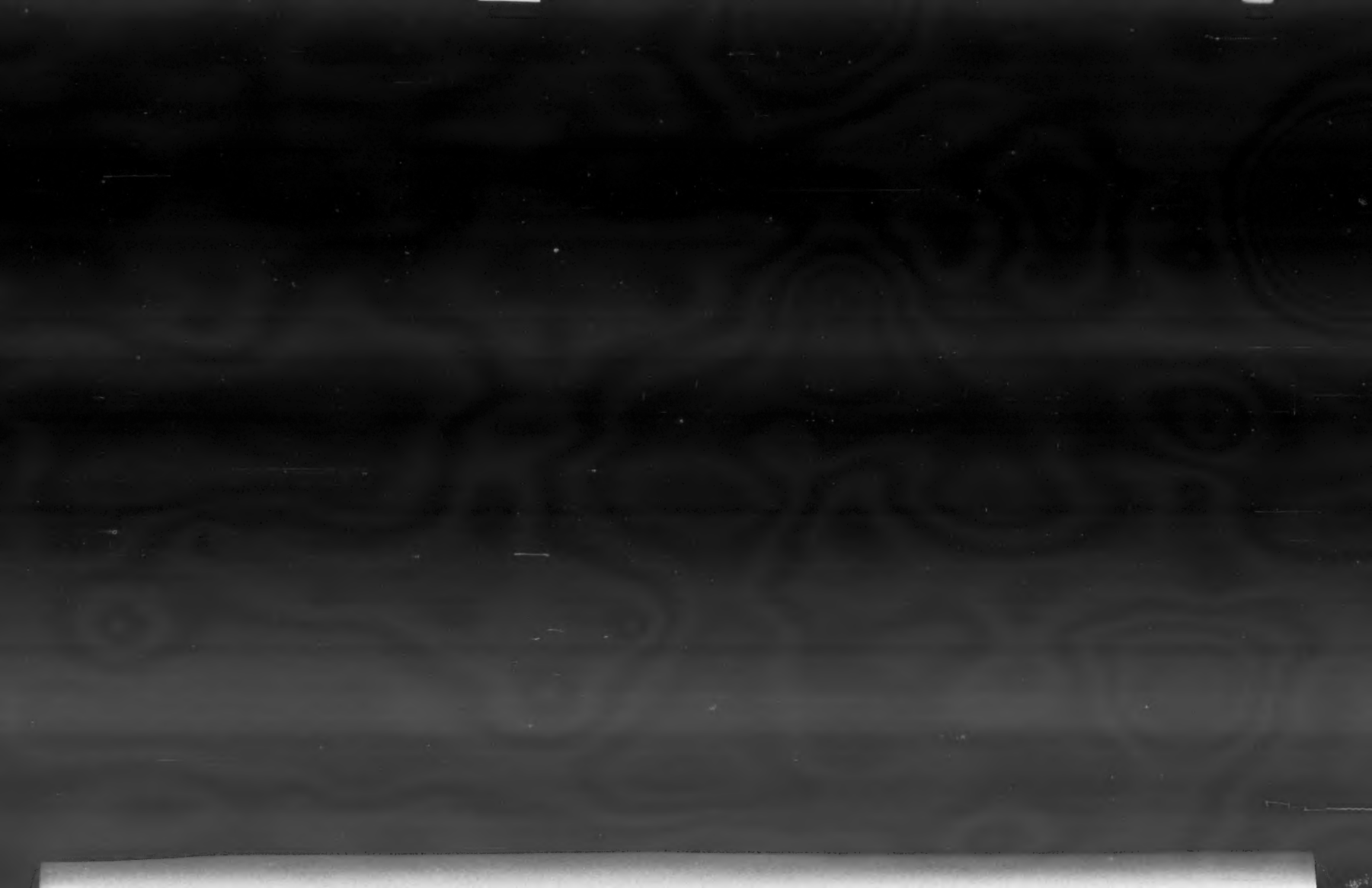
FIG. 9. Case 1. Whorls of cells are similar to those seen in Figure 7, but in solid masses.

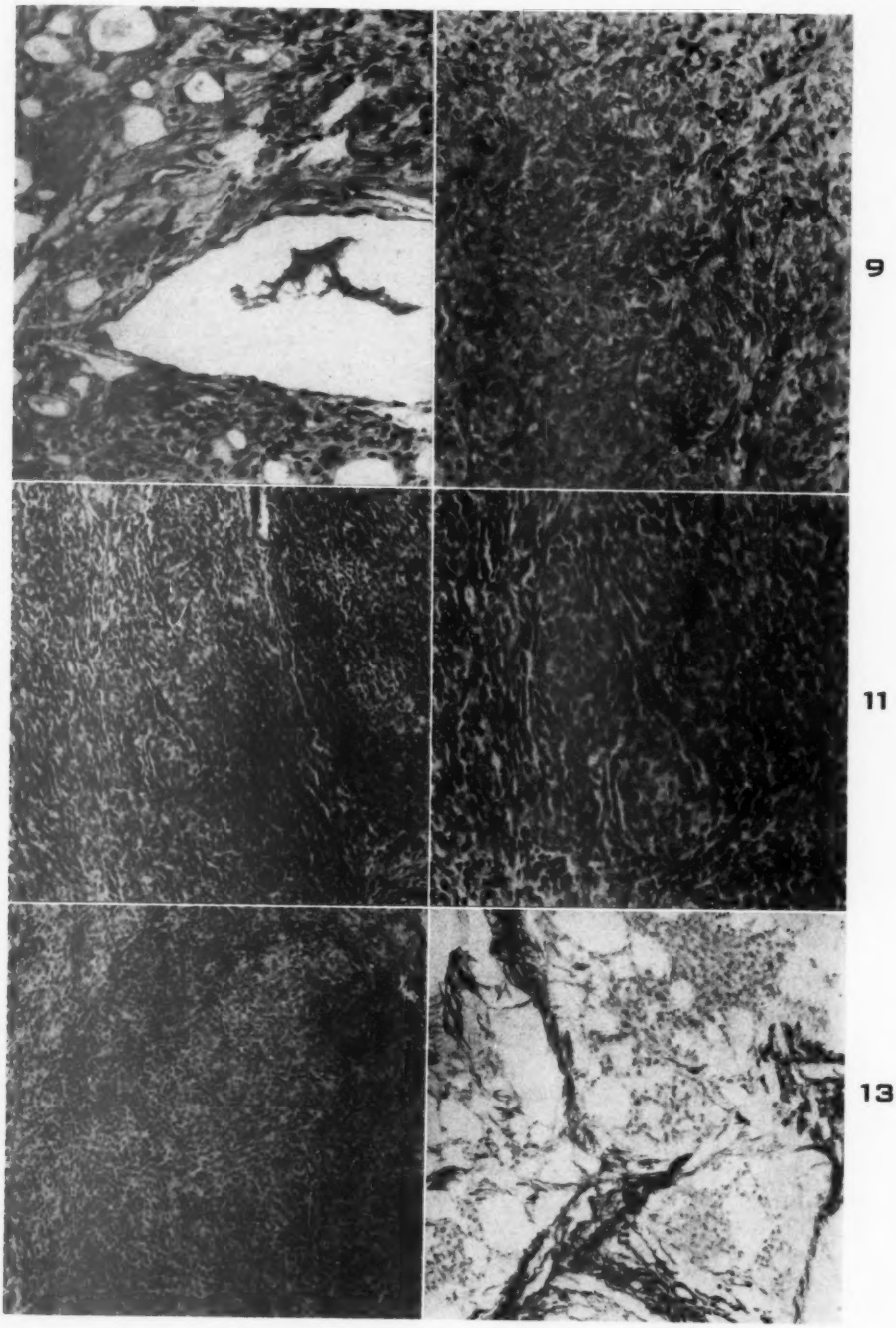
FIG. 10. Case 1. Groups of cells resemble those of Figure 9 in a heavy lymphoid stroma. This is from tissue resembling that below the trabecula shown in Figure 4.

FIG. 11. Case 1. Detail of Figure 10. The arrangement of cells vaguely suggests Hassall's corpuscles, but nowhere is there evidence of keratinization, nor are intercellular bridges present.

FIG. 12. Case 1. Within the lymphoid tissue these cells become less plump and are gradually lost among the lymphocytes.

FIG. 13. Case 1. Laidlaw's reticulum stain. Argyrophilic fibers do not support the individual cell but are confined to the fibrous stroma.





Hubbell and Liebow Lymphangioendothelial Thymic Tumors

PLATE 48

FIG. 14. Case 2. Posterior-anterior roentgenogram of chest showing mass projecting to the left of the mediastinum.

FIG. 15. Case 2. Lateral roentgenogram demonstrating anterior mediastinal position of the large mass outlined with the help of diagnostic pneumothorax.

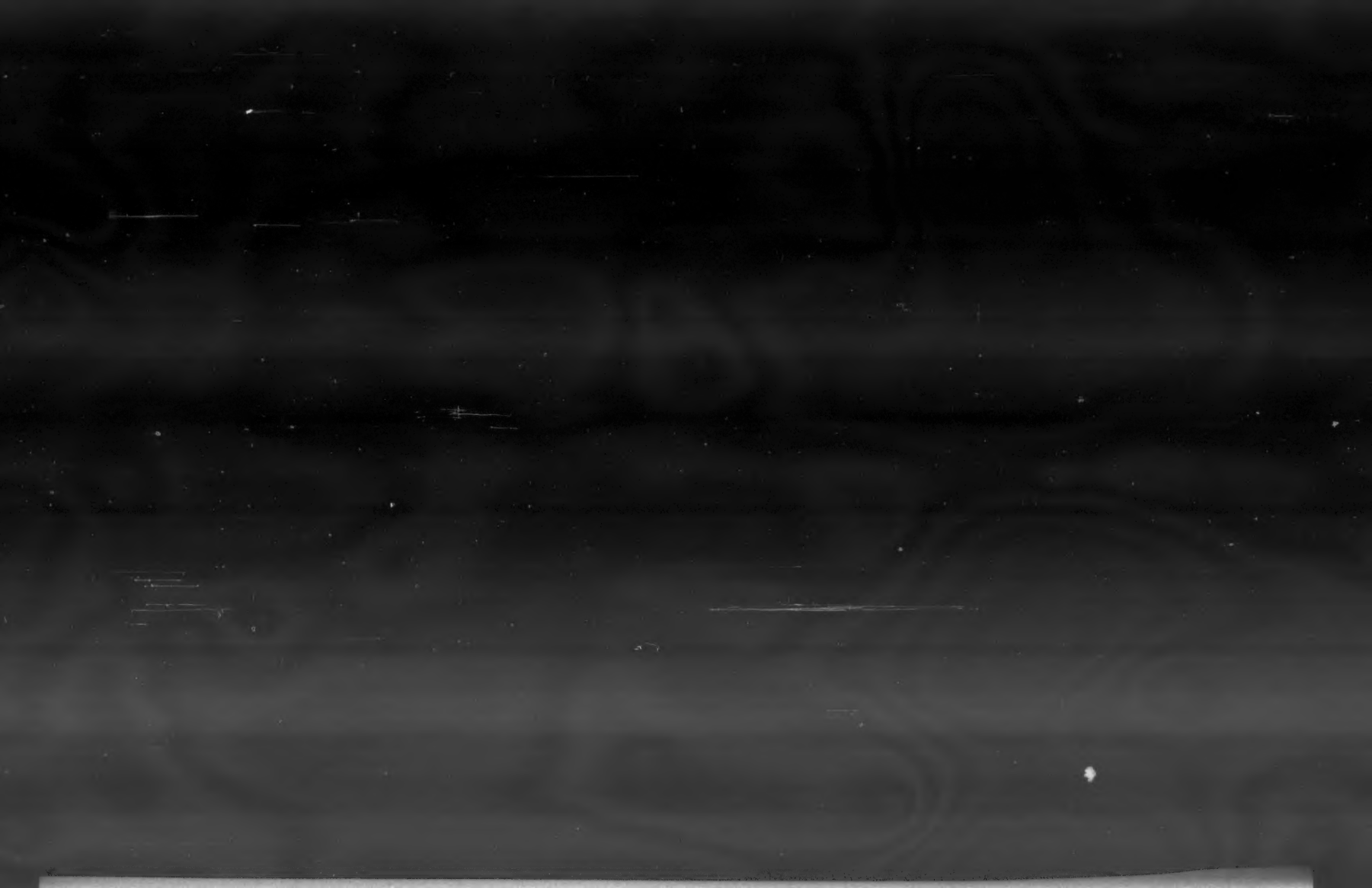


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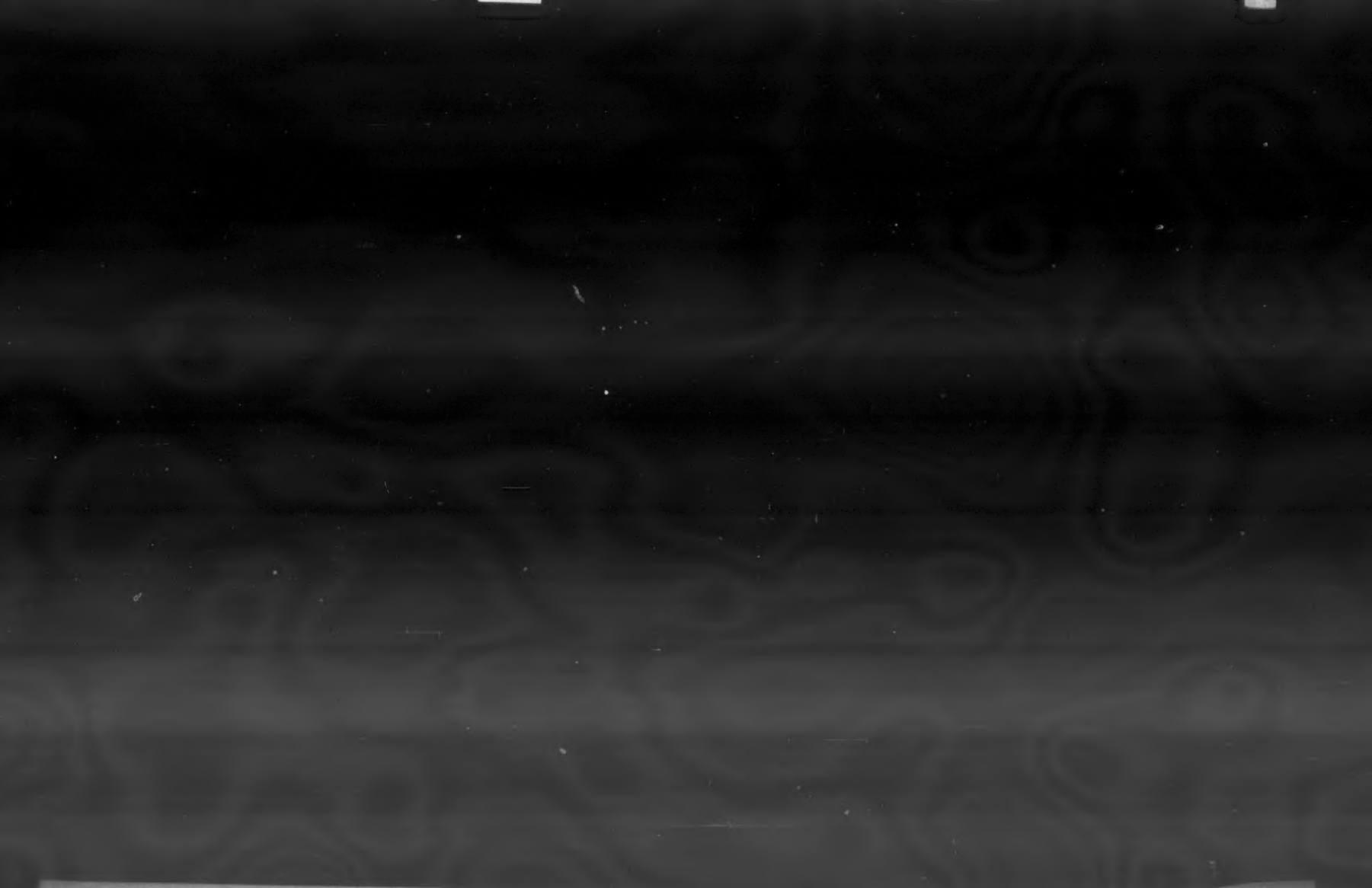
PLATE 49

FIG. 16. Case 2. External view of gross specimen showing the thin, well vascularized capsule.

FIG. 17. Case 2. Cut surface of the tumor showing a somewhat variegated appearance. There is hemorrhage near the upper pole and foci of necrosis are visible as white spots.

FIG. 18. Case 2. General view of the tumor, showing masses of lymphocytes at the left and, at the right, channels surrounded by spindle-shaped cells.

FIG. 19. Case 2. The spindle-celled component is shown here under higher magnification. The cells are arranged in intertwining masses, or concentrically about spaces that are lined by such cells.



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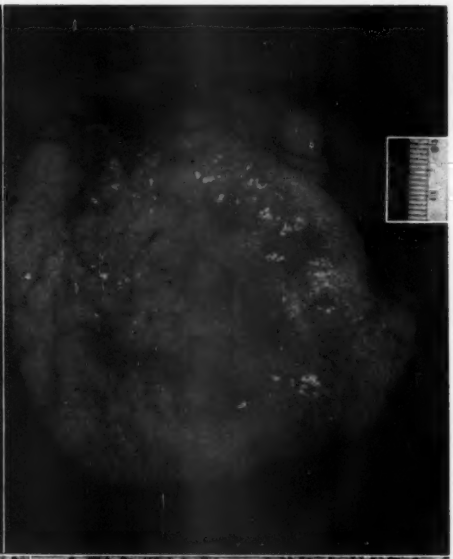

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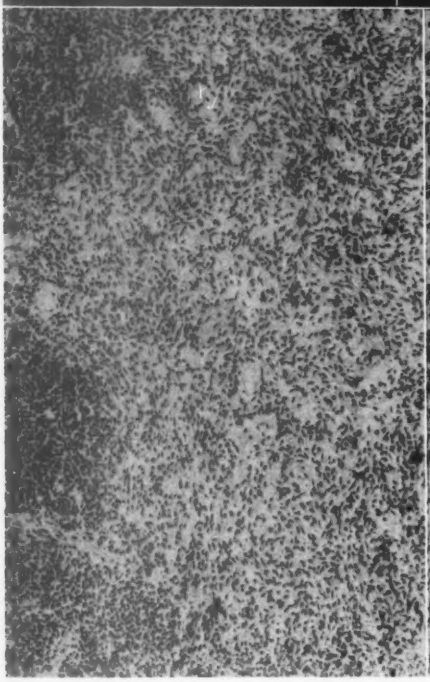

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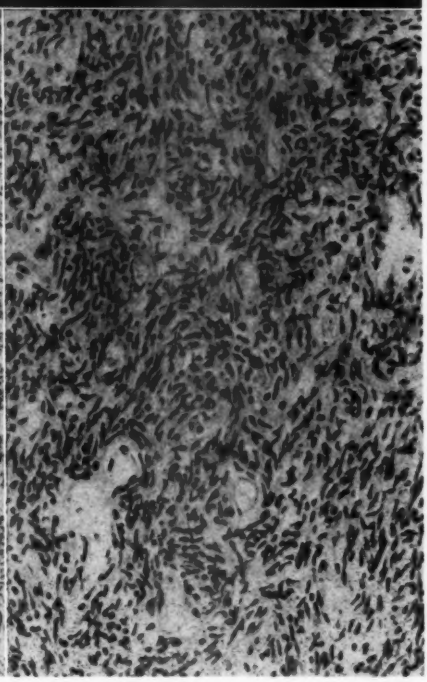

Hubbell and Liebow Lymphangioendothelial Thymic Tumors

PLATE 50

FIG. 20. Case 2. There is a sclerosing process about some of the presumably lymphoid channels, while others appear as in Figure 19. Here lymphoid cells in considerable number accompany the spindle-shaped cells.

FIG. 21. Case 2. A large lymphatic space is lined in part by the spindle cells and in part by more cuboidal plates probably derived from the former. There are many lymphocytes among the spindle cells.

FIG. 22. Case 2. A field of proliferated cells interpreted as lymphangioendothelium forming a compound channel as in case 1 (Figs. 4 to 7).

FIG. 23. Case 2. Laidlaw's reticulum stain. There are moderate numbers of argyrophilic fibers among the spindle cells and lymphocytes in this field, while elsewhere the reticulum is scanty.

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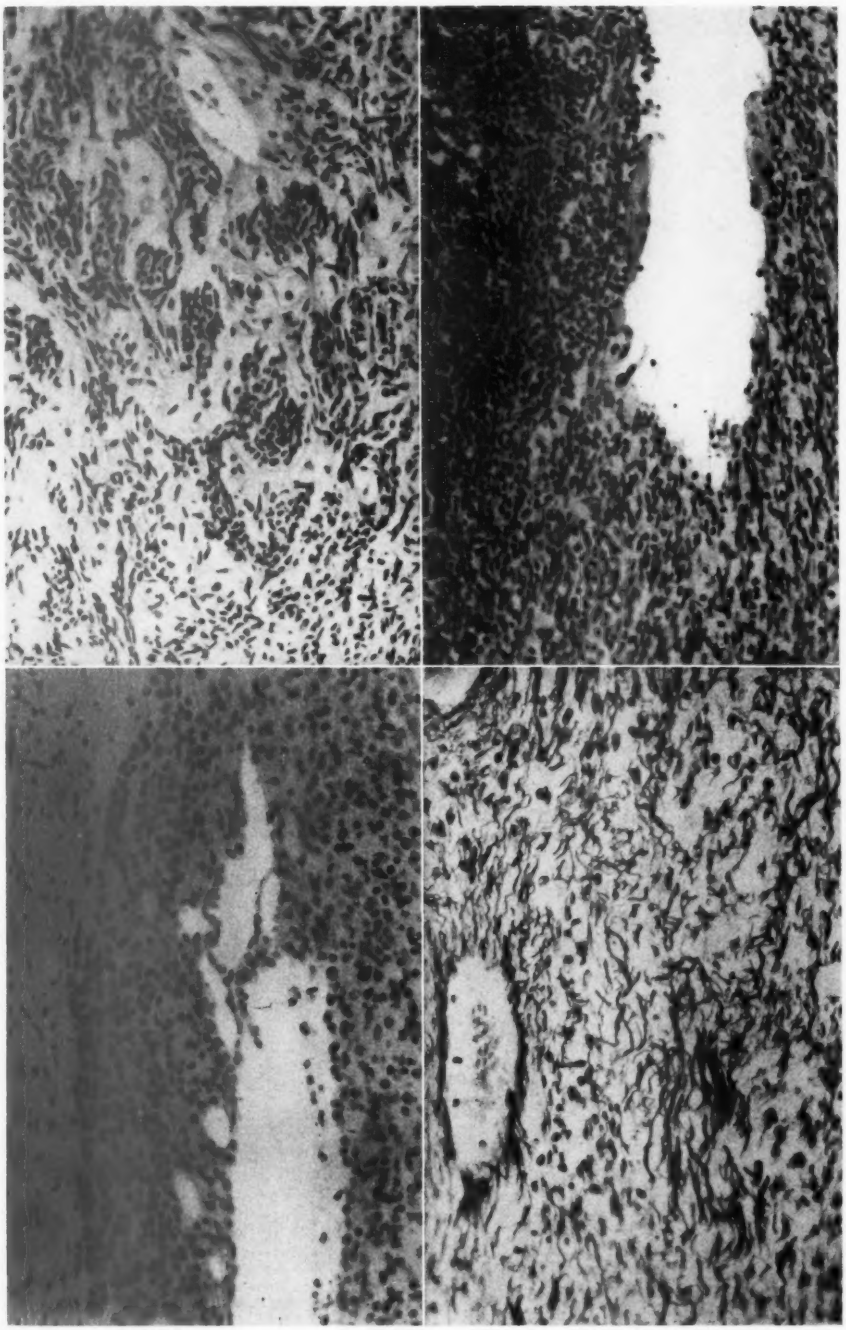

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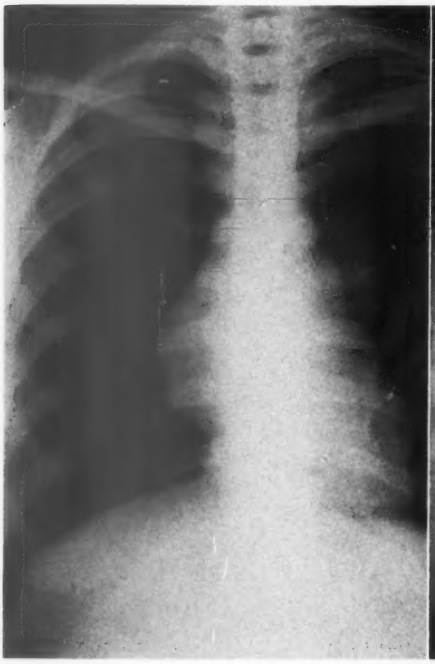
Hubbell and Liebow Lymphangioendothelial Thymic Tumors

PLATE 51

FIG. 24. Case 3. Posterior-anterior roentgenogram. A convex shadow is seen extending to the right of the cardiac density.

FIG. 25. Case 3. Lateral roentgenogram demonstrating the anterior mediastinal position of the mass.

FIG. 26. Case 3. Roentgenogram taken following a diagnostic pneumothorax, with patient lying on her right side. The mass swings away from the cardiac shadow.



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Hubbell and Liebow Lymphangioendothelial Thymic Tumors

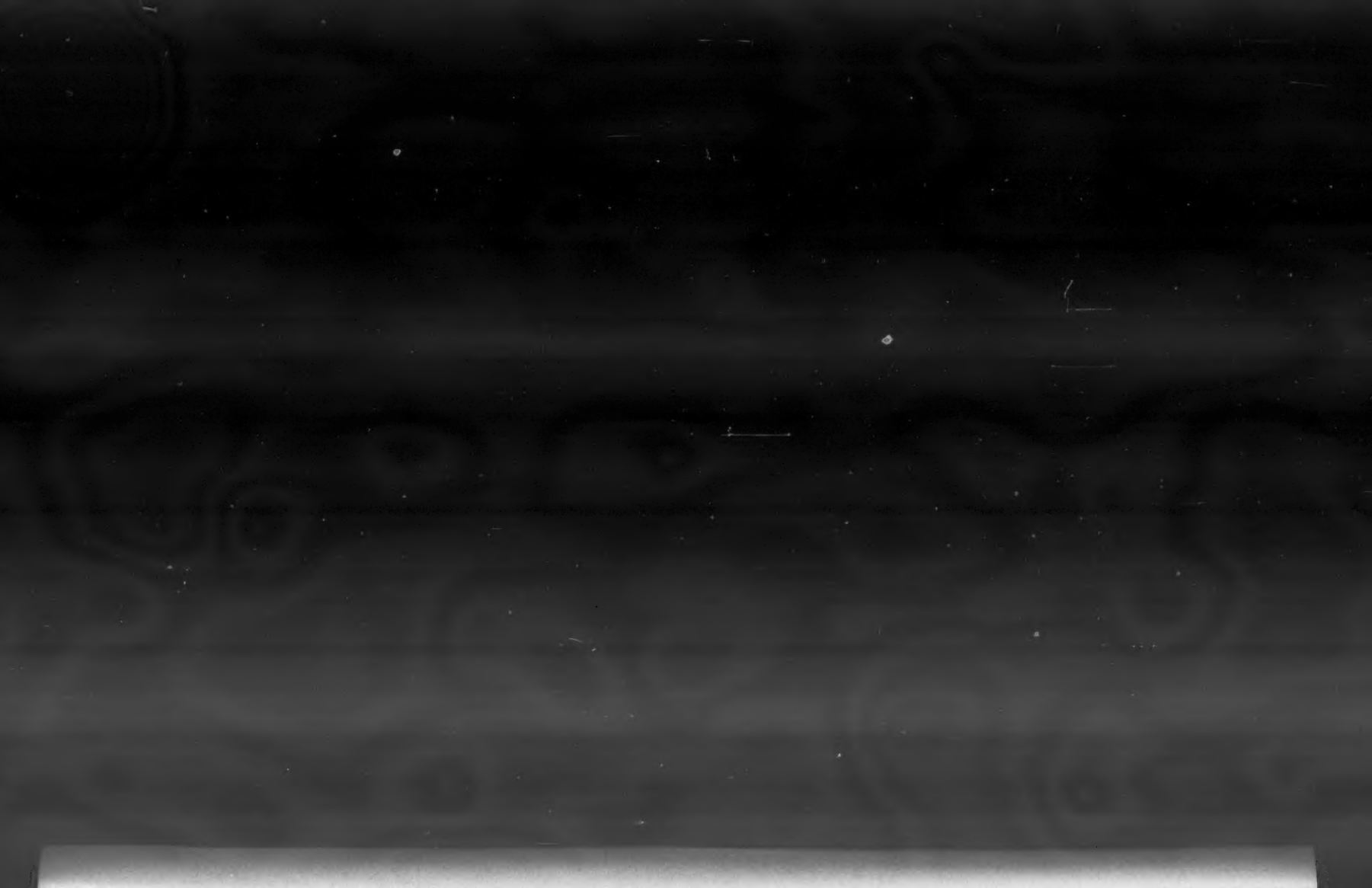
PLATE 52

FIG. 27. Case 3. Anterior aspect of mediastinal mass. There is a long upward protrusion into the superior mediastinum.

FIG. 28. Case 3. Cut surface. A large cavity has been opened, exposing encephaloid masses of soft gray tissue that protrude into the lumen. The clear fluid contents of the cyst have been lost.

FIG. 29. Case 3. In the upper portion of the field is shown a Hassall's corpuscle. Two channels are lined by flattened cells, one containing homogeneous acidophilic material.

FIG. 30. Case 3. A portion of the mass with the structure of normal thymus except for the large size of a Hassall's corpuscle. There is massive keratinization and focal calcification of the material. Several small lymphatic spaces are seen in the stroma above the center.



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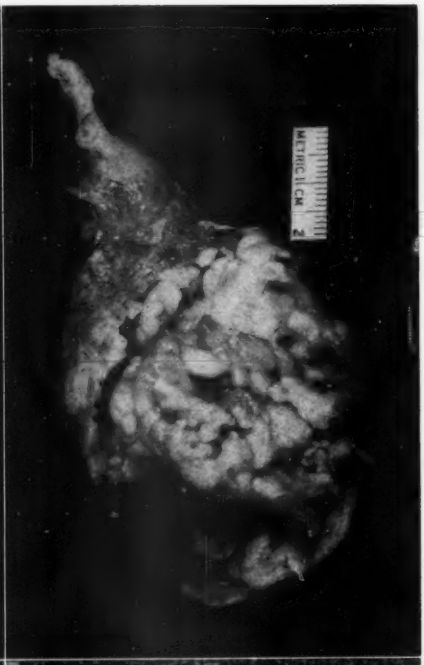

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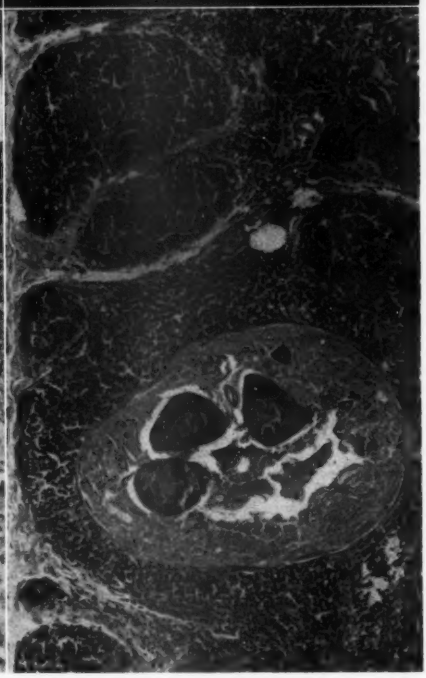

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Hubbell and Liebow Lymphangioendothelial Thymic Tumors

PLATE 53

FIG. 31. Case 3. Ramifying cavernous channels form the microcystic portion of the tumor.

FIG. 32. Case 3. Higher magnification of the large channels, demonstrating the lining of flattened cells and the collagenous walls. Numerous lymphocytes are present in the looser connective tissue among the channels.

FIG. 33. Case 3. Spindle-celled component of the tumor. Minute spaces occur among some of the fascicles of the cells. The structure here is similar to that seen in the other tumors of this series (see, especially, Figs. 12 and 21).

FIG. 34. Case 3. Laidlaw's reticulum stain showing scanty framework of argyrophilic fibers.

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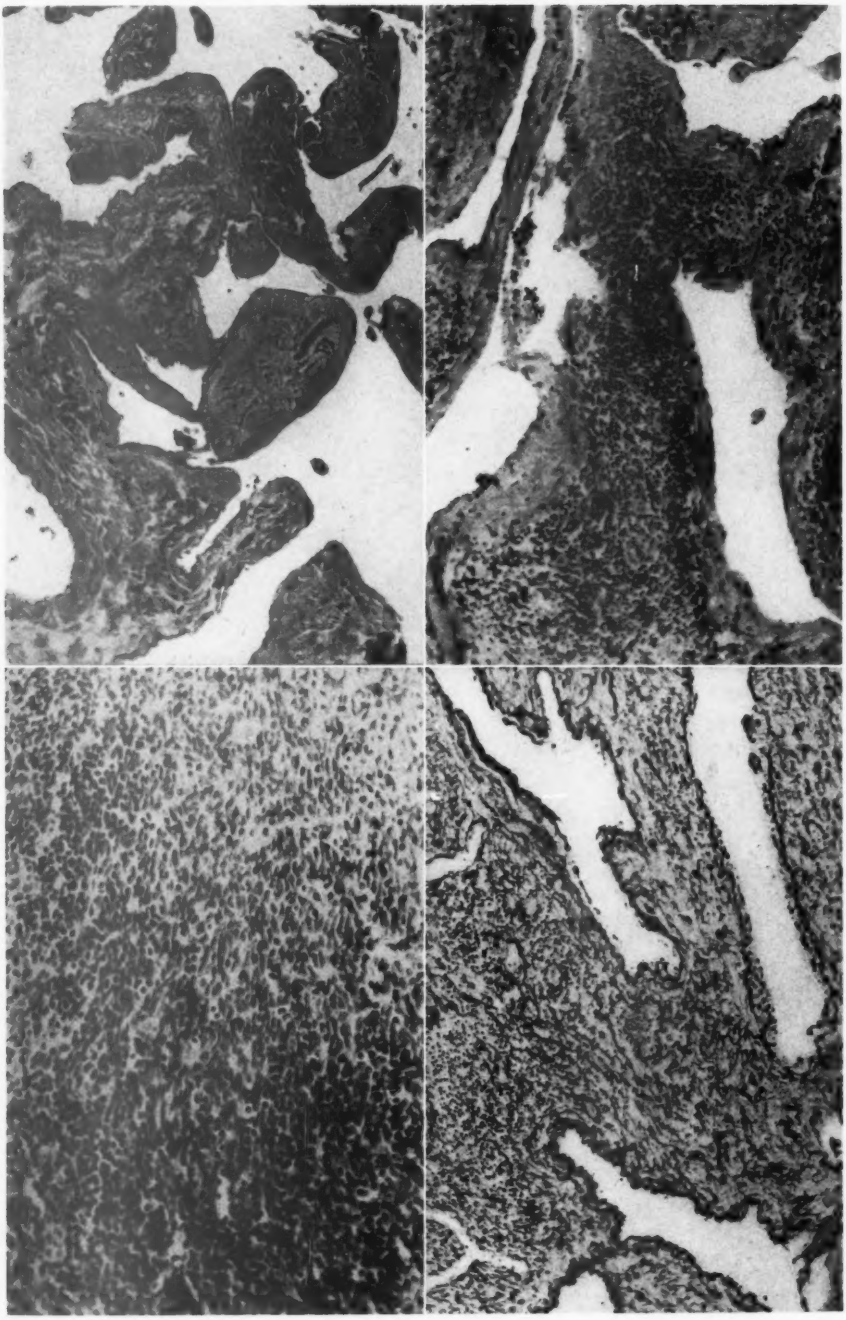

Hubbell and Liebow Lymphangioendothelial Thymic Tumors

SPONTANEOUS DEMYELINATING DISEASE OF ADULT RATS*

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(From the Department of Bacteriology and Immunology, Harvard Medical School, Boston 15, Mass.)

Spontaneous demyelinating encephalomyelitis of virus etiology (JHM virus) in mice has been described in previous papers from this laboratory.[1,2] Recently, we have had occasion to study a spontaneous paresis of adult rats with similar extensive demyelinating lesions of the spinal cord. As yet, we have not been successful in transmitting the disease to other rats or to mice, and thus the viral etiology of this disease remains unproved. Although no new cases have developed in our stock and further investigation for the present is halted, it nevertheless seems worth while to place our observations on record because of the very unusual character of the lesions which are unlike any previously described in this species.

The 2 adult rats in which the disease occurred were the third generation born of mothers obtained from a commercial dealer. During the first 7 weeks, they were maintained in a room in which there had previously been no mice. They were then transferred to another room in which a colony of mice were kept. For at least a year, however, there had been no animals infected with JHM virus in this room. They were given a presumably complete diet of purina fox chow.

When the rats were about 9 weeks old, it was noted that they were dragging their hind legs, and in the course of the next few weeks, the paresis became progressively more severe. The forelegs seemed to be less affected. There were no tremors, ataxia, or other evidence of encephalitis, and their general condition, save for moderate malnutrition, probably due to the difficulty in obtaining food, remained good. The paralysis never became complete; although locomotion was accomplished by dragging the body with the forelegs, the rats, when stimulated, were still able to perform irregular movements with their hind limbs.

Several other rats of the same litter and kept in the same cage developed no signs of illness.

The rats were killed with ether when they were respectively 84 and 105 days old. The brain and the spinal cord with its vertebral investment were fixed in Bouin's fluid, the viscera in Zenker's solution. Sections of the cord were taken at all levels, embedded in paraffin, and stained with hematoxylin and eosin, and with Mahon's[3] myelin stain.

* Aided by a grant from the National Foundation for Infantile Paralysis.
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Blood serum was obtained from the second rat and stored in the deep freeze. A segment of the lower cord was dissected out under sterile precautions, ground in buffered saline solution, and frozen for inoculation experiments.

Pathologic Findings

Significant lesions were confined to the spinal cord, and were similar in the 2 rats. As shown in Figures 1, 2, 3, and 4, there was found to have occurred almost complete destruction of the myelinated lateral and ventral columns. In the younger rat, the lower sacral segments of the cord were still almost intact, but in the older animal, the degenerative changes were found at all levels. The dorsal columns were affected to but a slight degree in the cervical cord, and not at all in lower segments. A narrow band of myelinated fibers contiguous to the central gray matter had escaped destruction.

In the degenerated white tracts, the loss of myelin was attended by the formation of ragged cysts, some of large size. It was impossible in preparations stained with hematoxylin and eosin or for myelin to detect any neuraxons in these cystic areas; they appeared to have undergone dissolution. However, in Bodian preparations of longitudinal sections of the cord, it was seen that the neuraxons, for the most part, were well preserved in the demyelinated tracts, though pushed apart by the swollen or degenerated myelin (Fig. 5). This would explain the fact that paralysis was not complete in the lower extremities.

There was complete absence of inflammatory reaction, either in the meninges or cord. However, there were occasional fatty granule cells, and slight increase or condensation of microglial elements in some areas.

The ganglion cells and other elements of the gray matter were normal at all levels (Fig. 6). So, too, were the dorsal and ventral nerve roots, the dorsal root ganglia, and the sciatic nerve.

The brain, including the medulla, pons, and cerebellum, was free from encephalitic or demyelinating lesions. In this respect, this spontaneous disease of rats differs from that caused in mice or young rats by injection of JHM virus.

No lesions of any significance were found in the thoracic or abdominal viscera. It will be recalled that focal lesions of the liver very frequently occurred in mice infected with JHM virus, but not in young rats.

Attempts at Passage

In an attempt at passage, 0.02 cc. of a 5 per cent suspension of fresh spinal cord from the second rat (no. 4799) was injected intracerebrally into 8 mice, 3 weeks old, and into 7 suckling rats, 4 to 5

days of age. The mice showed no signs of disease, and were discarded after having been observed for 22 days. The suckling rats also failed to develop any signs of illness. Two of them were sacrificed for histologic study after 2 weeks; brain, cord, and viscera were found to be normal. The remaining 5 rats have now been under observation for 2½ months, and seem entirely well.

The attempt to establish a viral etiology has thus failed. Because of the long duration of the illness and the chronicity of the lesions, it is of course possible that the original virus was no longer present when the rats were killed. Until it shall become possible to obtain material from rats in the early stage of the disease, the question must remain unanswered.

Attempt to Demonstrate Neutralizing Antibodies Against JHM Virus in the Serum of Rat 4799

Although the direct attempt to demonstrate virus in the spinal cord of one of the affected rats met with failure, it was of interest to ascertain whether the serum obtained at death might contain neutralizing antibodies against the JHM virus. If they were found, it would at least suggest an antigenic relationship between the agents causing the rat and mouse disease respectively. The following experiment, however, offers no evidence in favor of such relationship. Three sera were tested for possible neutralizing properties: (1) serum 6512, obtained from paralyzed rat 4799 at time of necropsy; (2) serum 7511, taken from an apparently normal cage-mate of rat 4799; (3) normal guinea-pig serum. All sera were inactivated at 56° C. for 30 minutes, diluted 1:1, 1:10, and 1:100, and mixed with equal volumes of JHM virus suspension, diluted so as to contain approximately 10 LD_{50} in 0.03 cc. These mixtures were injected intracerebrally into weanling mice after 1 hour of incubation at 37° C. Results are shown in Table I.

Table I

Results of Test for Neutralization of JHM Virus

Serum	Dilution	Animals infected
6512	1:1	6/6
	1:10	6/6
	1:100	5/5
7511	1:1	6/6
	1:10	6/6
	1:100	6/6
Guinea-pig	1:1	6/6
	1:10	6/6

The complete absence of neutralizing antibodies in the serum of rat 4799, taken at least 6 weeks after the onset of the paralysis, makes it unlikely that the JHM virus was concerned in the production of the lesions.

SUMMARY

In 2 adult rats which spontaneously had developed almost complete paraplegia, there was found extreme destruction of the ventral and lateral myelinated tracts of the spinal cord. The neuraxons, however, were preserved. The dorsal columns were but slightly affected. The central gray matter was intact, and no lesions were found in any part of the brain. The thoracic and abdominal viscera were not significantly altered.

Efforts to transmit the disease to young rats or mice were unsuccessful. No neutralizing antibodies against the demyelinating JHM virus of mice could be demonstrated in the serum of a rat obtained 6 weeks after the paralysis of the hind limbs was first noted.

I wish to acknowledge my indebtedness to Miss Sheila Richardson for technical assistance.

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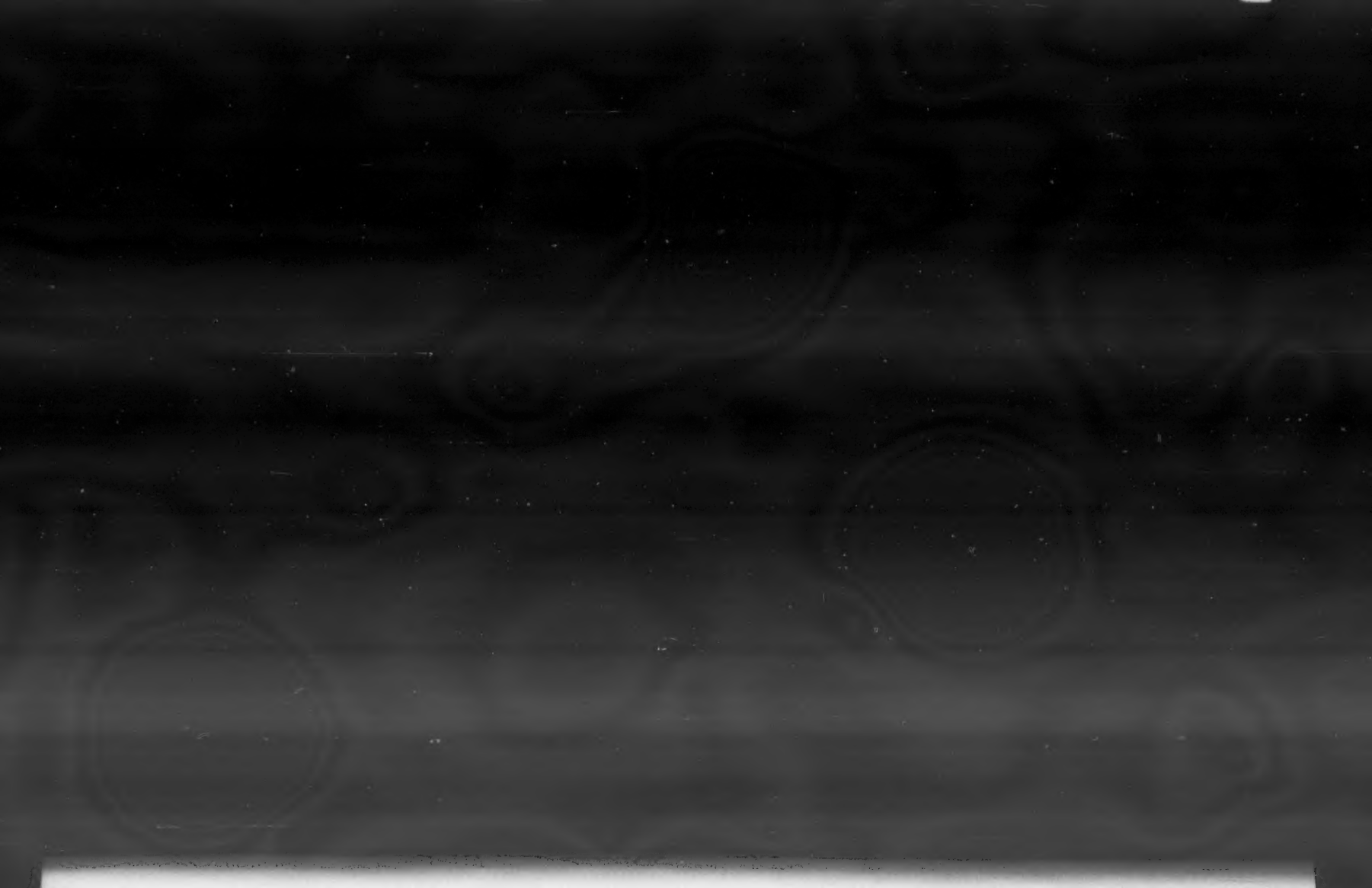
DESCRIPTION OF PLATES

PLATE 54

FIG. 1. Rat 4799. Age, 105 days. Cervical spinal cord. Demyelination of ventral and lateral tracts and partial demyelination of posterior column. Nerve roots and posterior spinal ganglia are intact. Mahon's myelin stain. × 38.

FIG. 2. Rat 4799. Dorsal cord. Mahon's stain. × 48.



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Pappenheimer Demyelinating Disease of Adult Rats

PLATE 55

FIG. 3. Rat 4765. Age, 84 days. Lumbar cord. Mahon's stain. × 42.

FIG. 4. Rat 4765. Sacral cord. Slight demyelination in ventral tracts only. Mahon's stain. × 42.

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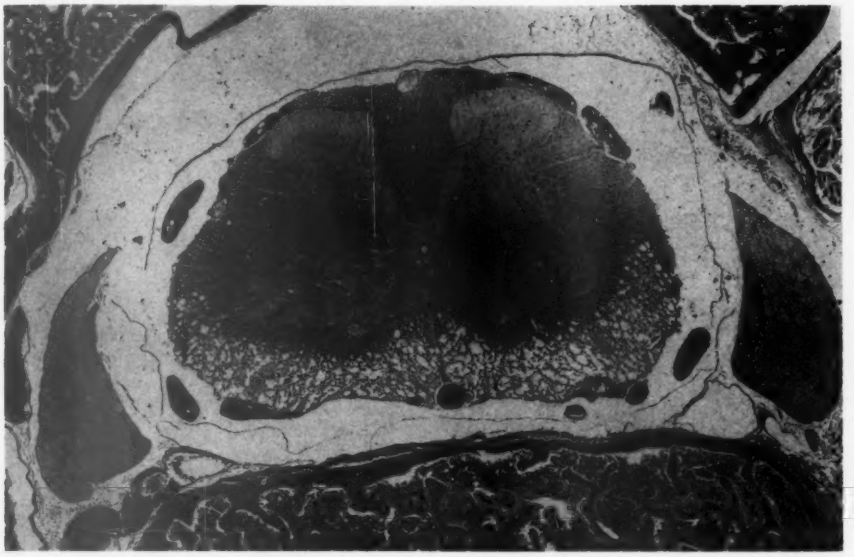

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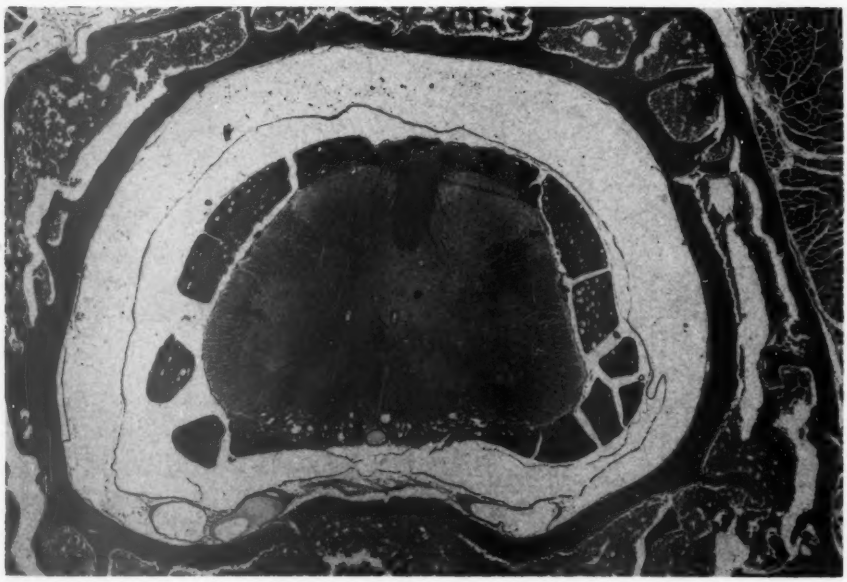
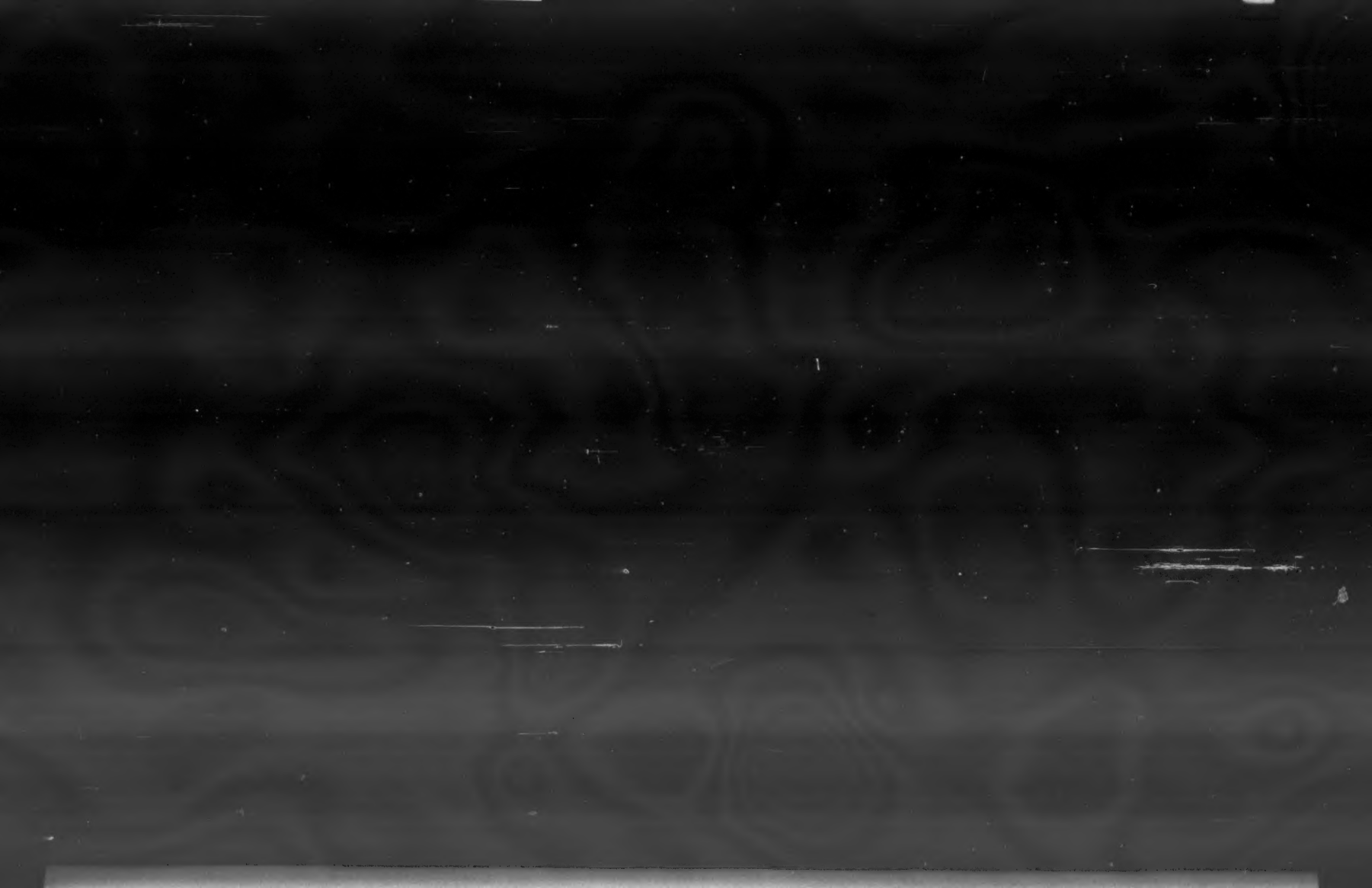


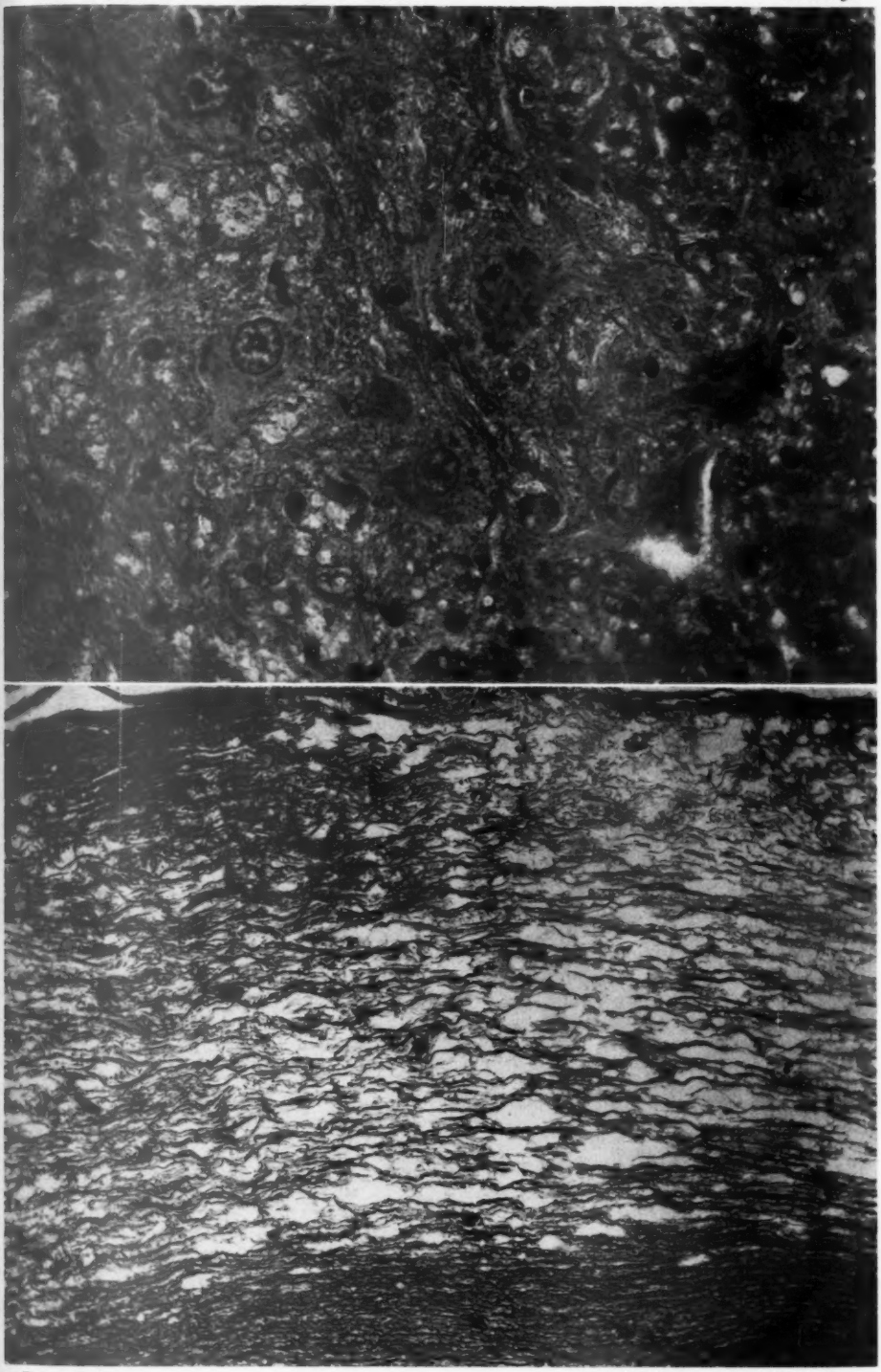
Pappenheimer Demyelinating Disease of Adult Rats

PLATE 56

FIG. 5. Rat 4799. Anterior horn. Intact ganglion cells and absence of inflammatory reaction. Hematoxylin and eosin stain. × 525.

FIG. 6. Rat 4799. Longitudinal section of spinal cord, showing preservation of neuraxons in demyelinated tract. Bodian's stain. × 120.





Pappenheimer Demyelinating Disease of Adult Rats

A STUDY OF GLIOBLASTOMA MULTIFORME BY THE GOLGI METHOD *

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Most of our knowledge of the gliomas has been gained with modern metallic impregnation technics. However, with these methods we may be overlooking considerable morphologic detail; indeed, we may be in error concerning the actual cellular components of these tumors. The present study represents an attempt to evaluate the ability of the Golgi method to provide more information about the composition of gliomas. The Golgi method was chosen because of its unique position among histologic technics. It gives, when successfully applied, the most complete visualization of the cellular elements of the nervous tissue, and in the past it has demonstrated the fundamental histologic details of such complex structures as the retina and cerebellum.

In only three publications is reference made to attempts to study the glial tumors by the Golgi method. Golgi[1] himself described several brain tumors and with his technic revealed their glial nature. Rosenthal[2] used the Golgi method for study of a glial tumor in the spinal cord. Hortega[3] applied his modification of the Golgi method to one example of astroblastoma. He expressed his results by stating that he had had an opportunity to apply to one tumor his modification of the Golgi method and that this gave very illustrative cellular pictures. He stated further that the lack of material had prevented the further application of this new modification concerning which he had great expectations. Unfortunately, death (1945) interrupted his brilliant work.

Eleven tumors with the histologic diagnosis of glioblastoma multiforme were available for my study. Sections of these tumors were stained satisfactorily and the results are presented. These include several new observations.

MATERIALS AND METHODS

Several known modifications of the Golgi method were tried first, but it soon became evident that the best method for study of glial elements was Hortega's modification (Hortega,[4] Cajal and De Castro[5]).†

* This investigation was supported by a research grant by the Cancer Research Society of Montreal.

Received for publication, October 12, 1951.

† I am indebted to Dr. R. W. Graves for additional information concerning the employment of this method.

The following technic was finally adopted:

1. The fresh tissue received in saline solution from the operating room was sectioned into blocks 3 to 4 mm. thick and placed in the following solution for varying intervals of time (see results):

Potassium bichromate	5 gm.
Chloral hydrate	3 gm.
Formalin (40%)	10 cc.
Distilled water	90 cc.

(Potassium bichromate was dissolved in water, formalin added, then finally chloral hydrate. The solution has to be freshly prepared before use.)

2. The blocks were washed rapidly in 3 or 4 changes of distilled water. The blocks intended for longer fixation were washed rapidly in 10% formalin and then transferred to a fresh bichromate solution.

3. Four small dishes, each containing 25 cc. of 1% silver nitrate and one flask containing 100 cc. of 1.5% silver nitrate solution were prepared. The blocks were then gradually transferred from one silver solution to another, remaining in each for approximately 1 to 3 minutes, and then collected in the flask containing 1.5% silver nitrate. The blocks of tissue were handled with paraffin-coated forceps. The number of blocks per 100 cc. of silver nitrate or bichromate solution did not exceed five.

4. After silver impregnation for 18 to 24 hours, the tissue blocks were rinsed in 3 or 4 changes of distilled water and brushed free of precipitate.

5. Frozen sections were cut at intervals of 60 to 100 μ and collected in 30% alcohol. The tissue blocks should be frozen gradually and not too hard.

6. Dehydration: The sections were then transferred to a second 30% alcohol, 95% alcohol, absolute alcohol, and finally to toluol.

7. The sections were transferred from toluol onto the slides, blotted with filter paper, mounted in abundant permount,* and covered with coverslips. The preservation of Golgi preparations presents a difficult problem. However, at the present time there is still no trace of deterioration to be seen in our permount sections, some of which are 10 months old.

As a histologic control for the Golgi preparations, additional blocks of the same tissue were stained with several metallic and chromatic stains. In this way the histologic nature of the tumor was verified in each case.

* Obtained from the Fisher Scientific Co., New York, N.Y.

Results

A characteristic feature of the Golgi method is the selective staining of different cellular elements that can be obtained by varying the period of fixation in bichromate solution. This feature was confirmed in the present study. The period of fixation in bichromate solution influenced the selective staining of cellular elements in glioblastoma as follows:

After a fixation period of 10 to 12 hours, the less differentiated forms of the spongioblastic series were the cells that were predominantly stained, whereas astroblasts and astrocytes were infrequently stained. The tumor elements were best seen (and in their true proportions) after 16 to 24 hours of fixation in bichromate solution. Reacting microglia, which in the resting phase seems to require a much shorter period of fixation, could also be seen after this period of fixation. Following 24 hours of fixation, astrocytes and astroblasts were conspicuous, whereas the spongioblasts were seen only occasionally.

Cell Types

Examination of neoplastic tissue by the Golgi method confirmed the pleomorphic feature of glioblastoma multiforme. The great variety of cell forms and the gradual transition of one form to another frequently made it difficult to classify the neoplastic elements as belonging to specific types. The bulk were small cells of the spongioblastic type with intricate ramifications of their protoplasmic expansions (Figs. 4 and 11). Unipolar or bipolar spongioblasts without secondary branches (Figs. 2 and 3) were seen only infrequently. Sections fixed for a period of 12 to 16 hours demonstrated a poorly differentiated, small, round cell with a nipple-like pole (Fig. 1). This cell was thought to resemble the primitive neuro-ectodermal cell of Deery[6] or the migratory spongioblast of Penfield.[7] The constant presence of these cells in considerable numbers was evidence against their being artefacts resulting from the plane of section.

Astroblasts and their transitional forms were difficult to identify. Cells with the following features were classified as belonging to the astroblast series: (1) cells of a size larger than spongioblasts; (2) cells showing more than two primary expansions; (3) cells possessing vascular attachment more often than spongioblasts (Fig. 5).

The study of neoplastic astrocytes was not pursued since the distinguishing features of a neoplastic astrocyte are uncertain.

The presence of giant forms of spongioblasts, astroblasts, and astrocytes was a conspicuous feature in several of the glioblastomas studied.

In giant astrocytes it was possible to follow the gradual loss of abundant fine expansions; at the same time some of the processes became conspicuous through their thickness (Figs. 6 and 7). It is worth while to mention that cells of almost identical nature were observed in one specimen of tuberous sclerosis, which was available for study. This may be evidence in support of the view of Globus and Strauss,[8] who described several examples of glioblastoma multiforme accompanied by tuberous sclerotic changes.

Degenerative Changes

Small necrotic areas were observed to be sharply delineated in the Golgi preparations and these were usually filled with amorphous cellular débris (Fig. 11).

Pseudopalisading was a conspicuous feature and was represented by the expansions of neoplastic elements projecting perpendicularly toward the necrotic areas (Fig. 12).

Contrary to the conclusions of Hardman[9] that the vessels in the deeper parts of glioblastoma multiforme are formed by alteration of pre-existing capillaries, my study with the Golgi preparations revealed that new capillary formation takes place. Pictures similar to those shown in Figure 10, demonstrating the growth of individual endothelial cells, frequently were seen in the vicinity of central necrotic areas. Closer to the necrotic zone, many cells that were morphologically identical with vascular endothelial cells appeared to be without any recognizable connection with vascular channels.

In the majority of tumors studied there was extensive microglial reaction, which, as could be expected, was centered chiefly around areas of tissue disintegration. The resting forms of microglia were infrequently seen. On the other hand, large numbers of transitional forms could be identified. The retraction and thickening of microglial expansions were accompanied by the presence of delicate spines on the surface. Some of these spines showed globoid swellings, with a tendency to become confluent, and in this way the transition to the ameboid form seemed to occur.

The areas of increased microglial activity provided an opportunity to confirm Penfield's[10] observation of dendrophagocytosis. Astrocytes surrounded by reacting microglial cells were seen frequently. In many instances the microglia seemed to be applied to the astrocytic expansions or to their fragments (Fig. 9).

In areas of microglial activity an interesting phenomenon was observed, which can best be named moniliform change of neoplastic glial elements. This change consisted of the presence of multiple bead-like

swellings of the cellular expansions. It affected different forms of the spongioblastic and astroblastic series (Fig. 13) and may be compared with the change in astrocytes described by Hortega[11] in certain forms of intoxication.

Border Zone

It is commonly accepted that glioblastoma belongs to the diffusely infiltrating tumors, which show poor demarcation from the surrounding brain tissues. Astrocytic gliosis, however, is frequently present in the zone of infiltration and sometimes it may amount to a dense glial barrier. In our Golgi preparations, astrocytic proliferation in the border zone of the tumor was a common finding. It was interesting to note, however, in a few tumors studied, that oligodendroglia also seemed to be involved in an attempt at fibroglial delimitation. In these areas oligodendroglial cells were large, with hypertrophic expansions and gliosomes (Fig. 14), while the astrocytes frequently showed some moniliform swelling of their expansions.

Summary

The technic of Golgi impregnation was employed to study neoplastic glial elements. It was applied in 11 cases of glioblastoma multiforme. The pleomorphic features of this neoplasm were confirmed; as well as some phenomena within the tumor such as pseudopalisading, new formation of capillaries, reacting microglia with dendrophagocytosis and moniliform change. Astrocytic proliferation was a common finding in the border zone of the neoplasm and participation of oligodendroglia in the attempt at glial delimitation was noted.

I wish to acknowledge my indebtedness to Dr. W. V. Cone for suggesting this investigation, and for advice and encouragement during its pursuit.

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DESCRIPTION OF PLATES

PLATE 57

FIG. 1. The cell corresponding to the migratory spongioblast of Penfield. Fixation period, 14 hours. × 1172.

FIG. 2. Two unipolar spongioblasts. Their position suggests direct division. Fixation period, 18 hours. × 1172.

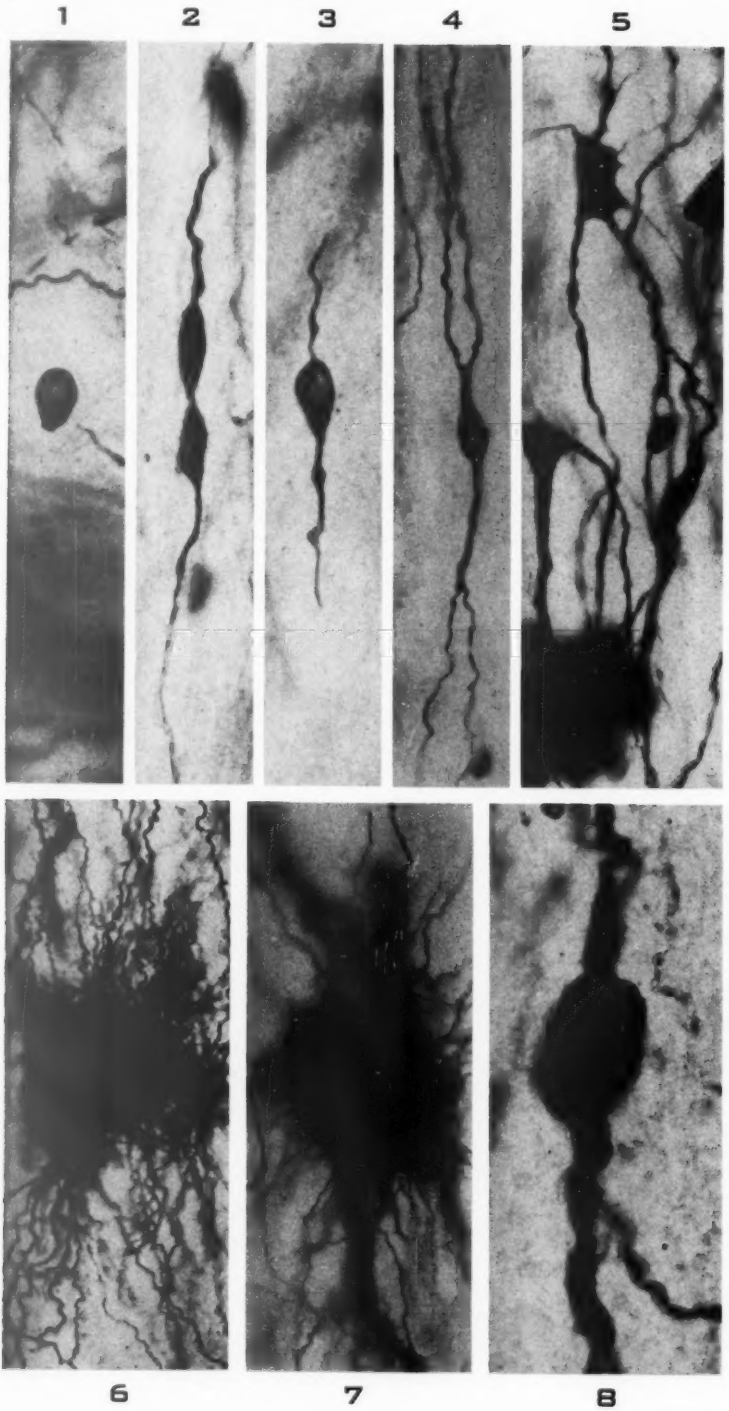
FIG. 3. Bipolar spongioblast. Fixation period, 16 hours. × 1172.

FIG. 4. More differentiated bipolar spongioblast. Fixation period, 18 hours. × 1172.

FIG. 5. Astroblasts with vascular attachments. Fixation period, 24 hours. × 1172.

FIGS. 6 and 7. Two giant astrocytes showing thickening of some processes and gradual loss of fine expansions. Fixation period, 12 hours. × 1172.

FIG. 8. Giant bipolar spongioblast. Fixation period, 12 hours. × 1172.



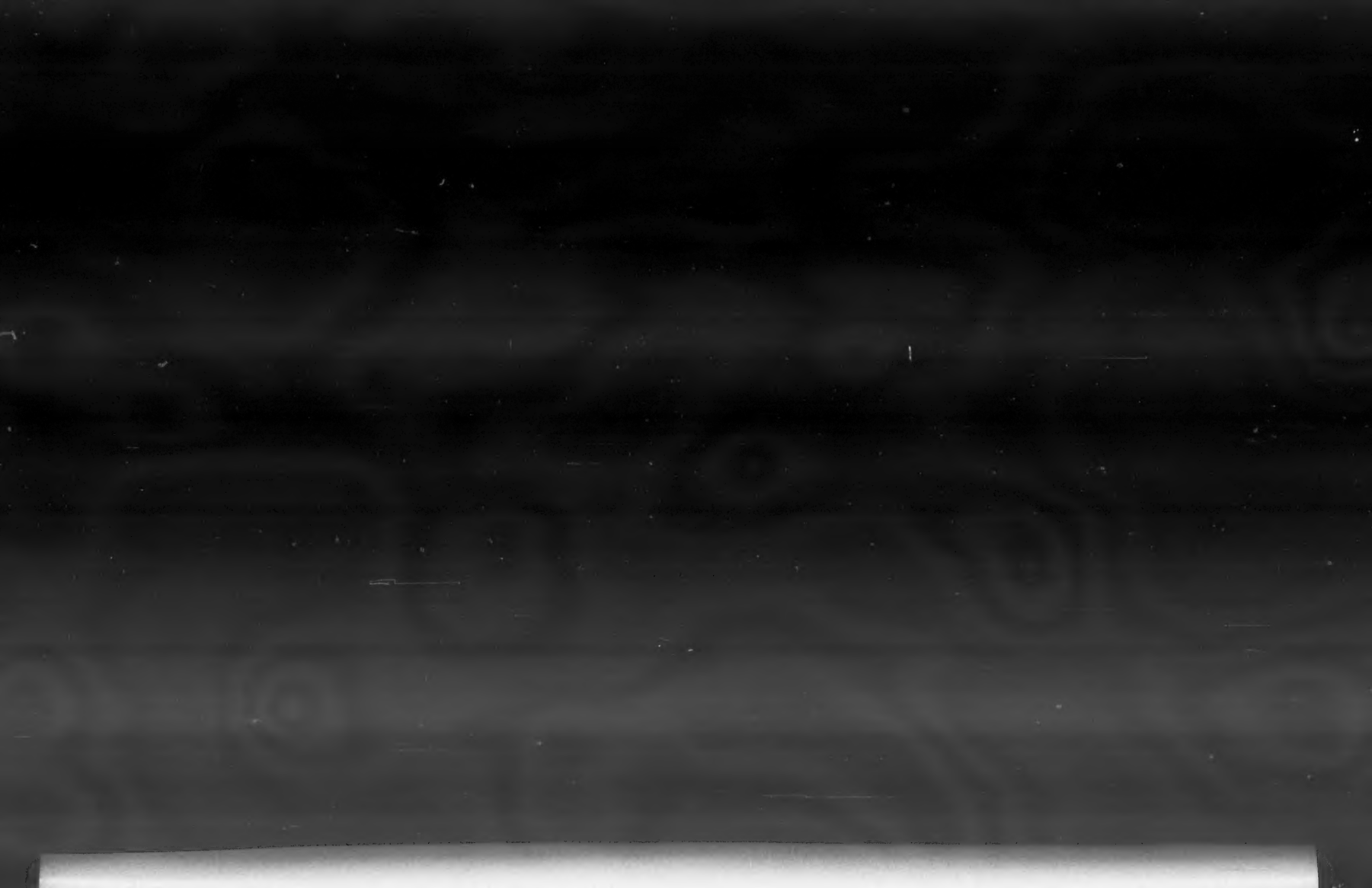
Klatzo

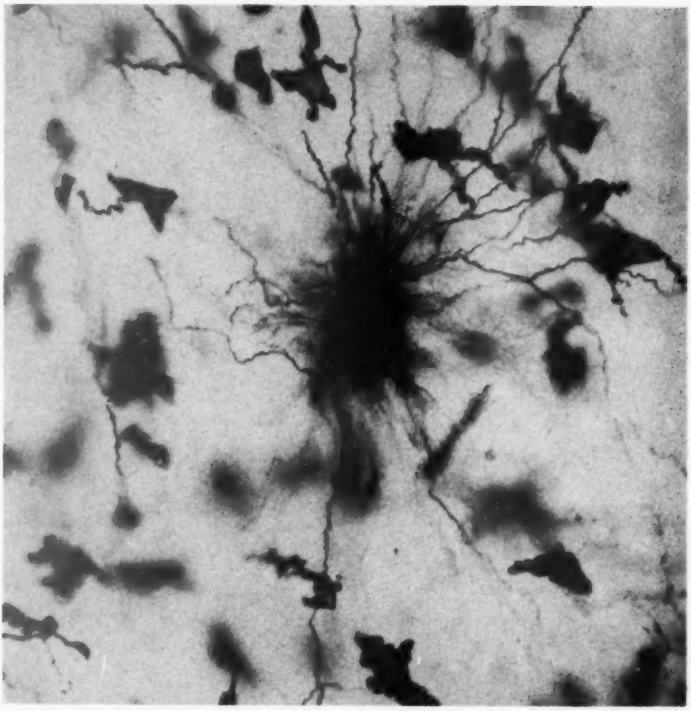
Glioblastoma Multiforme

PLATE 58

FIG. 9. Dendrophagocytosis. Reacting microglial cells surrounding an astrocyte. Some of the ameboid cells engulf the astrocytic expansions, some only their fragments, while some of the cells apparently lie free. Fixation period, 20 hours. × 680.

FIG. 10. New formation of capillaries. Fixation period, 18 hours. × 680.





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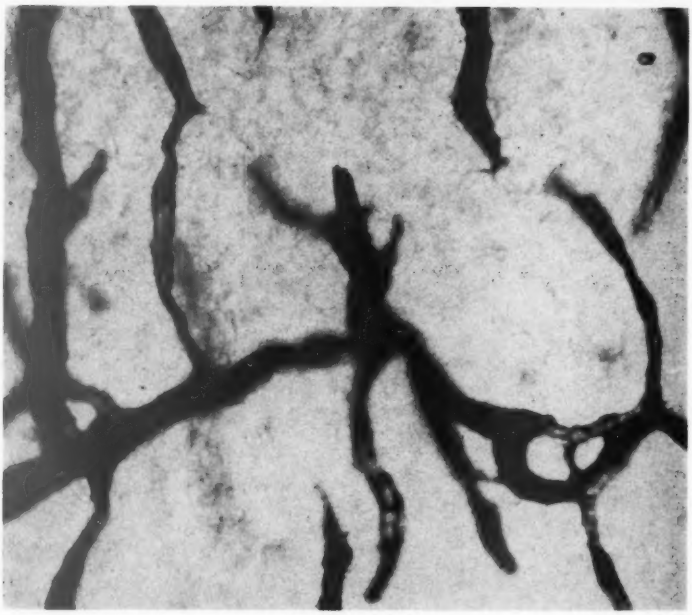

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Klatzo Glioblastoma Multiforme

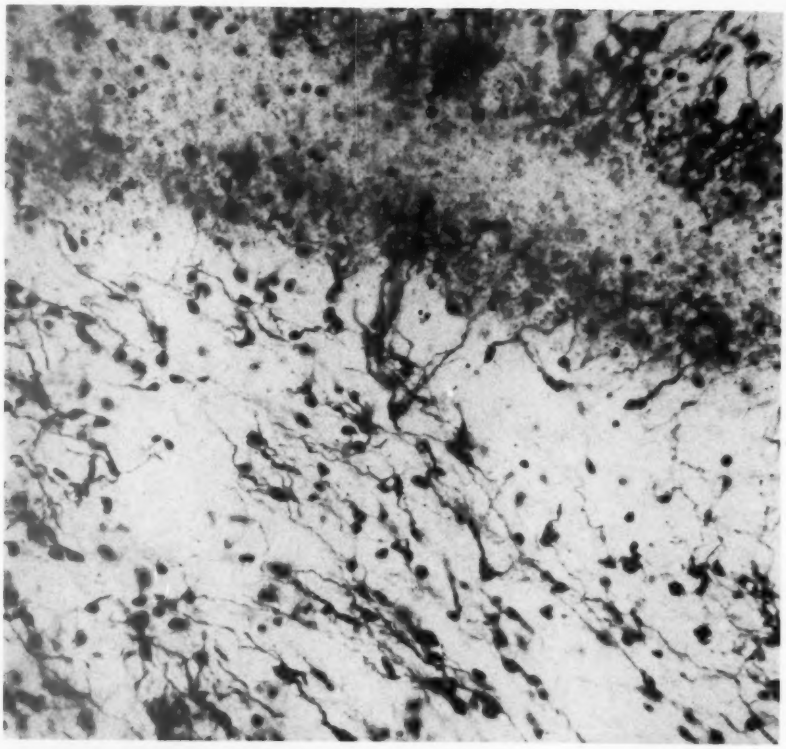
PLATE 59

FIG. 11. Small necrotic area. Pleomorphic neoplastic elements, predominantly of the spongioblastic and astroblastic series. Fixation period, 12 hours. × 284.

FIG. 12. Perpendicular orientation of expansions toward necrotic center in pseudopalisading. Fixation period, 12 hours. × 680.

FIG. 13. Astroblast undergoing moniliform change. Fixation period, 20 hours. × 680.

FIG. 14. Border zone. Hypertrophic oligodendrocytes with enlarged gliosomes. Fixation period, 18 hours. × 1172.



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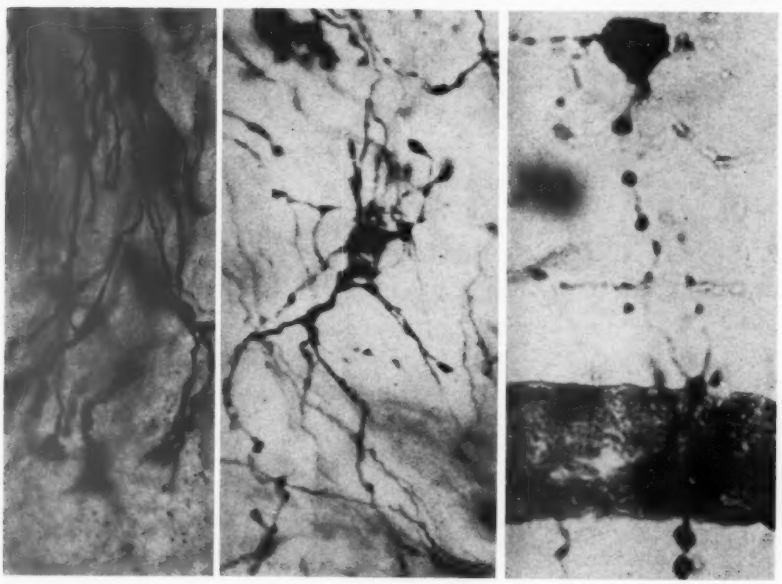

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Klatzo Glioblastoma Multiforme

FAMILIAL DIABETES MELLITUS IN MICE, ASSOCIATED WITH INSULIN RESISTANCE, OBESITY, AND HYPERPLASIA OF THE ISLANDS OF LANGERHANS *

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Over a half century has elapsed since von Mering and Minkowski[1] reported the production of diabetes mellitus in dogs by extirpation of the pancreas, but the cause and nature of the naturally occurring disease remain obscure. Warren[2] has expressed the pathologist's difficulties in attempting to explain this disease process: "Few diseases definitely associated with any one function, metabolic or otherwise, show as wide a range of concomitant pathological change as does diabetes—or as frequent absence of demonstrable pathological change." Bell[3] was able to demonstrate marked reduction, or even absence, of beta granules in the pancreatic islands in only 63 per cent of patients with diabetes mellitus. Warren[2] found no demonstrable pathologic changes in the islands of Langerhans in 20 per cent of 842 patients with diabetes. In the remainder, however, he was able to demonstrate hyalinization of islands, hypertrophy of islands, fibrosis or lymphocytic infiltration of islands, hydropic degeneration of cells, pyknosis of the nuclei, sometimes hemochromatosis, as well as an occasional distinct reduction in the number of islands. Recent experiments summarized by Cori[4] indicate the importance of an antagonistic pituitary factor in carbohydrate metabolism. However, Eisenhardt and Warren[2] found no consistent lesions in the pituitary glands of 17 diabetic patients, although 4 exhibited focal necrosis; one, basophilic adenomata; one, variation in cell size; and one, vacuolation of chromophobe cells.

While it is established that there is a hereditary factor in the occurrence of diabetes,[5] the importance of this factor is not clear. Similarly, the etiologic significance of the association of diabetes with obesity[6] is not established.

A small group of mice with familial diabetes mellitus and associated defects has recently been established. These diabetic animals constantly exhibit a striking insulin resistance, centripetal obesity, and certain tissue changes. It is the purpose of this report to describe the characteristic morphologic abnormalities in these mice and to compare

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them with their non-obese, non-diabetic siblings and parents, and with normal Swiss mice. A number of important correlations with pancreatic, pituitary-adrenal, and metabolic factors will be made.

Material and Methods

Mayer and co-workers[7,8] have recently reported the appearance in V strain mice, and in a hybrid group of mice derived from a cross of V strain and fuzzy animals, of a form of obesity inherited as a simple mendelian recessive. These obese mice have blood sugar values generally exceeding 300 mg. per cent in the non-fasting state, and glycosuria on the order of 3 per cent. They are hyperglycemic even after the administration of 400 units of insulin per kg. Fasting causes 40 to 50 per cent reduction in blood sugar. Adrenalin causes marked rise in blood sugar values. These mice choose a high fat diet on free selection of nutrients. Non-obese siblings of the obese animals are consistently killed by small doses (0.5 unit) of insulin and have normal blood sugar levels, which can be markedly elevated by adrenalin. Whether these non-obese sibs are heterozygous for the gene for obesity is unknown. Parent fuzzy mice are non-obese and normoglycemic.

Eleven obese mice and 13 non-obese siblings were available for study. Together with 8 mice of the parent, fuzzy strain and 7 Swiss mice controls, the animals were killed by jar etherization or by decapitation, generally at the ages of 3 to 5 months. Tissues were generally fixed within a few minutes. One obese animal died at the age of 7 months but was not received for dissection until 2 days later. Portions of skin, heart, lung, mediastinum, spleen, stomach, small and large intestine, pancreas, liver, adrenal gland, kidney, bladder, uterus, ovary, testis, epididymis, aorta, salivary gland, tongue, skeletal muscle, bone, thyroid and pituitary glands, mid-brain, spinal column, and bone marrow of representative animals were fixed in 4 per cent formaldehyde solution or Zenker's acetic fixative, sectioned at 6 μ after paraffin embedding, and stained with hematoxylin and eosin. Pancreas, liver, kidney, skeletal muscle, tongue, and skin were fixed in absolute alcohol and in Gendre's alcoholic acetic picroformalin fluid for Best's carmine and Schiff's periodic acid leukofuchsin stains for glycogen. Pancreases were fixed in Bouin's fluid, in Zenker-formol, or mordanted in Zenker's fluid after formalin fixation for Gomori's chrome alum hematoxylin stain and azocarmine-aniline blue-orange G stain, with and without permanganate oxidation, for pancreatic island cells. Adrenal glands were stained for fat with Sudan IV after frozen sectioning of formalin-fixed tissue. Pituitary bodies were stained with Mallory's phosphotungstic acid hematoxylin for demonstration of basophile granules.

Gross Findings

The obese animals, excepting one immature animal, ranged from 35 to 56 gm. in weight (Table I). A weight of 90 gm. was attained by one

Table I

Comparative Size and Number of Islands of Langerhans

Animal		Age	Weight	Number of islands		Size of islands in μ	
				Maximum per 100× field	Total of 10 fields	Maximum	Average of 10
		days	*gm.*				
Obese	067	53	29	6	32	171	77
	020	91	42	5	25	266	235
	080	97	35	6	16	320	160
	026	101	56	12	26	380	233
	030	103	40	4	9	256	154
	032	114	49	7	35	600	225
	031	115	51	6	30	450	213
	002	124	51	5	32	425	225
	057	160	48	6	32	550	243
	055	160	47	10	45	400	175
	088	175	49	7	44	400	214
Siblings	NoY	33	16	3	7	190	84
	440	53	19	6	18	500	182
	50	71	22	6	34	250	120
	N32	78	25	4	17	300	82
	760	84	21	6	25	247	152
	90	85	21	6	21	615	247
	790	91	22	5	11	513	184
	N31	114	23	5	25	300	115
	N71	115	26	4	13	235	120
	450	120	21	8	18	303	250
	090	126	24	10	16	247	152
	N23	180	31	3	9	225	123
	N77	268	29	2	4	675	341
Parents	BA	153-167	25	2	6	175	105
	BHr		25	2	6	225	116
	BAc		26	8	30	200	135
	BHa		25	3	7	200	95
Swiss	31	90-130	22	2	9	50	35
	23		20	3	14	95	55
	21		23	2	3	171	56
	24		21	2	3	143	80
	32		20	2	6	175	95
	22		20	3	15	200	99
	1		21	2	5	226	177

mouse which was not dissected. The non-obese siblings weighed from 21 to 26 gm., excepting 2 immature and 2 aged animals, as did the normal parents and Swiss mouse controls. The mice were all of similar length. The non-obese animals were well proportioned, but the obese mice exhibited striking central localization of fat, causing approximately 50 per cent increase of abdominal girth over normal, 25 per cent increase in thoracic girth, and 20 per cent increase of neck circumference. The size of extremities and facies did not appear abnormal. The testes of obese animals did not descend into the scrotum on

abdominal pressure, as they did in non-obese and control mice.

The hair of the non-obese animals was luxuriant. That of the obese mice exhibited inconstant thinning over the dorsal trunk. Two obese animals had actual cephalic baldness. A number of obese animals developed striking focal alopecia during the use of the feeding cannisters. These lesions were irregularly demarcated and extended over most or all of the squatting surface of the buttock and over wide expanses of body wall. The areas of denuded skin were lightly wrinkled, inelastic, parchment-thin in some portions, and covered with thin, adherent, gray-tan crusts or with dried blood in others. The central portions of many exhibited punched-out ulcers with bases of lobular, glistening, yellow-white subcutaneous fat crusted with blood, and ragged margins of thin skin (Fig. 1).

The subcutaneous fat of the obese animals was greatly increased in thickness. It measured as much as 0.6 cm. in thickness on the abdomen, 0.5 cm. on the chest, 0.3 cm. on the neck, and 0.3 cm. on the dorsal trunk, as compared with less than 0.1 to 0.2 cm. for non-obese and normal animals. The properitoneal fat was 0.7 cm. thick in obese mice. The fat was pale yellow to almost white, and was coarsely lobulated by thin, fibrous septa. The peritoneal and mesenteric depots were correspondingly large, but those around the heart and in the mediastinum were not remarkable.

The dispersal of the pancreatic tissue within the mesentery made measurements of the size of this organ inaccurate. But, with one exception, no gross difference between the pancreases of obese, non-obese, sibling and parent, and normal Swiss mice was noted. The exception was an obese mouse with definite translucence and whitening of the pancreas. Microscopically, the islands of Langerhans were preserved in this animal, but most of the acini had been replaced by fatty tissue and clusters of lymphocytes and histiocytes. No bile stones nor a probable cause of pancreatic duct obstruction was found in this animal.

In a few obese animals the livers were yellow-brown, compared to the usual chestnut brown. No evidence of renal scarring, infection, or contracture was noted. The large arteries did not appear sclerotic. The adrenal, thyroid, and pituitary glands of all mice were of similar weight and appearance. Other viscera contained no gross abnormalities.

Microscopic Examination

The skin and dermal appendages were generally well formed, but at the margins of the bald and ulcerated areas in the obese mice there was a gradual transition to thin epidermis with few or absent rete pegs and absence of dermal appendages. At the sites of crusting and ulcer-

ation there was abrupt transition to necrotic plaques of epidermis, or of epidermis and corium, or to complete loss of epidermis and necrosis of corium and subjacent fat and muscle. Some of the necrotic areas and many of the septa of the subjacent fibro-fatty and muscular tissue contained nests of polymorphonuclear neutrophils, eosinophils, and rare lymphocytes. Cutaneous blood vessel walls were of normal thickness. There was scattered neutrophilic infiltration of some of the cutaneous nerves, but no apparent fragmentation of nerve fibers was seen. In the necrotic, superficial tissues beneath the deep ulcers there were masses of moderately large cocci in great clusters; but at the area of transition from normal skin, no organisms could be seen. There was a surprising lack of epithelial, fibroblastic, or angioblastic proliferation near the ulcers.

The heart and lungs were not remarkable. No arteriosclerotic lesions were detected in these young animals. The livers of 2 obese animals contained a moderately large amount of lipid in small vacuoles within slightly enlarged cells throughout the hepatic lobules. No fibrosis or necrosis was noted in the livers of these, or of any other mice.

The islands of Langerhans were increased in number and in size in obese animals as compared with non-obese siblings, in non-obese siblings as compared with members of the parent strain, and in parents as compared with Swiss mice (Tables I and II). This was not a straight-line relationship. The differences of island size and number between obese and Swiss mice were statistically highly significant. The difference of island size between obese and parent mice was also statistically highly significant, but difference of island number between obese and parents was slightly significant. Differences of island size and number between obese and sibling mice were not statistically significant in these small series. With one exception, obese mouse 67, which was less than 2 months old and weighed only 29 gm., there were islands greater than 250 μ in diameter in the pancreases of all obese animals. In 6 of the 11 adult obese mice the maximum island diameter was 400 μ or more. In only 7 of the 12 adult non-obese siblings was the maximum islet diameter greater than 250 μ, and in only 4 was it greater than 400 μ. Conversely, in none of the parent or Swiss mice was an island with a diameter greater than 200 μ measured. Similarly, the average of ten island diameters was greater than 150 μ in all obese mice except obese mouse 67, and greater than 200 μ in 7 of 11. In only 7 of 12 adult non-obese siblings was the islet diameter more than 150 μ; in only 3 was it greater than 200 μ. Conversely, in the parent and Swiss mice, with one exception, the island diameters averaged less

TABLE II
Comparison of Size and Number of Islands of Langerhans

	I Obese (N=10) Mean	Em	II Siblings (N=12) Mean	Em	III Parents (N=5) Mean	Em	IV Swiss (N=7) Mean	Em	P values
Maximum number of islands per field at 100×	6.8	±.75	5.4	.62	3.8	1.44	2.3	.18	I vs II >.05 I vs III >.05 I vs IV <.01 II vs III >.05 II vs IV <.01
Total number of islands in 10 fields	29.4	3.6	17.6	2.0	12.2	6.3	7.9	1.9	I vs II <.01 I vs III <.05 I vs IV <.01 II vs III >.05 II vs IV <.01
Maximum diameter of islands, in μ	405	31.8	368	46.8	200	10.2	151	23.5	I vs II >.05 I vs III <.01 I vs IV <.01 II vs III <.01 II vs IV <.01
Average of 10 island diameters, in μ	208	10.3	172	21.5	113	8.6	85.3	17.6	I vs II >.05 I vs III <.01 I vs IV <.01 II vs III <.05 II vs IV <.01

N = number of animals in series.
Em = standard error of the mean.
P = probability value of difference between means (<.05 is significant; <.01 is highly significant).

than 150 μ. There was greater variation in size of islands in the obese mice. Many were located near pancreatic ducts. Occasional mitotic figures were found in islets of obese mice, and rare ones in non-obese siblings. Three of the obese mice had a definite trabecular pattern of island cells (Fig. 5) similar to that described in regenerating islets by Cecil.[9] That pattern was the only manifest cytologic difference between the islands of Langerhans of obese mice and of non-obese siblings (Figs. 5, 6, and 7). The alpha and beta cells of the pancreatic islets were similar in numerical proportion, granularity, size, character, and distribution in the obese, non-obese sibling, parent, and Swiss mice. Hyalinization, fibrosis, hydropic degeneration, or necrosis was not observed in the islands of Langerhans. The pancreatic acini and ducts in all but one animal were intact.

No glomerulosclerosis, vacuolation of renal tubular epithelium, or pyelonephritis was observed. The adrenal glands were examined carefully under survey and fat stains. With these basic technics the secretory zones were all similar to those in the Swiss mice. No cortical nodules were found. The adrenal medullae were not manifestly hypertrophied. Apparently normal, active spermatogenesis was found in both obese mice and non-obese siblings. The ovaries of both contained many well formed graafian follicles. The pituitary bodies of the obese mice, of siblings, parents, and Swiss mice contained basophils and acidophils in comparable numbers. No adenomatoid collections of cells, necrosis of cells, Crooke's changes of the basophils, variations in cell size, basophilic "invasion" of the posterior lobe, nor hyaline plaques could be found in the pituitary glands of these animals. The central nervous system presented normal structure and well preserved neurons at the hypothalamic, spinal cervical, and low dorsal levels.

There were characteristic deviations of glycogen content in the experimental animals. Sections of liver, stained for glycogen, permitted accurate differentiation of obese mice from all non-obese animals (Figs. 2, 3, and 4). The obese mice consistently exhibited much less hepatic glycogen than did all the others, although they showed a small to moderate amount. On the other hand, the non-obese siblings and parents showed slight to moderate increase of hepatic glycogen when compared with Swiss mice. In no cases were excessive amounts of glycogen, or abnormal amounts of nuclear glycogen observed. The glycogen was scattered throughout the lobules, occasionally concentrated in centrolobular and peripheral areas. Pilot chemical assays confirmed these quantitative abnormalities in hepatic glycogen. Skeletal muscle contained quite variable amounts of carminophilic glycogen. Very little glycogen could be stained in the skin of any of the animals.

No significant amount of glycogen was stained in the kidneys of the experimental or control mice.

Peripheral eosinophile cell counts were comparable in obese, sibling, parent, and control mice and revealed similar reduction after ACTH administration.

Comments

By the usual definition of diabetes, including hyperglycemia and glycosuria, the obese mice were diabetic. Although the blood sugar levels usually were taken in the non-fasting state, they indicated definite hyperglycemia as compared with the levels of control animals on diets with an even higher carbohydrate content.

Clinically we were dealing with two groups of animals: one group was obese and diabetic, and insulin resistant; the other group, including siblings, parents, and Swiss mice, was clinically normal. Morphologically, however, there were three groups of animals, in addition to controls: (1) obese, diabetic, insulin-resistant mice which had a marked increase in pancreatic island size and number and manifested marked decrease in liver glycogen content; (2) siblings, which were non-obese, normoglycemic, sensitive to insulin, revealed moderate increase of pancreatic island size and number, and had large amounts of liver glycogen; and (3) parents, which were non-obese, sensitive to insulin, normoglycemic, revealed slight increase of pancreatic island size and number in comparison with Swiss mice, and had large amounts of liver glycogen. It is not evident from this study just what interaction of hereditary and environmental factors produced these three types of animals; but clinically and morphologically the obese animals were in a group apart.

The obese animals manifested marked, statistically highly significant, increase in size and number of islands of Langerhans when compared with non-obese parents and Swiss mice. The numerical increase of islets was significant when compared with non-obese siblings, but the differences in islet size were only suggestive in these small series (Table II).

The islands of Langerhans in the obese mice, and to a less extent in the non-obese sibling and parent animals, exhibited many of the characteristics of islands reacting to a growth stimulus. They were enlarged and increased in number, mitotic figures were present, and many were clustered adjacent to ducts from which they are known to arise throughout life in other species of rodents.[10] Some of these islets had the trabecular pattern described by Cecil in regenerating islands.[9] The growth stimulus could conceivably be a primary pancreatotrophic principle; a secondary growth-stimulating factor, for example, a de-

mand for more insulin in the insulin-resistant organism; or a factor releasing inhibition of growth. We know too little of the checks and balances of island growth to delve deeply into the last possibility. The association of insulin resistance with marked island hypertrophy in the obese mice suggests a growth stimulus associated with insulin resistance. The correlation of marked island hypertrophy with hyperglycemia in the obese mice may indicate that the growth stimulus is related to hyperglycemia. Our methods were not sensitive enough to rule out slight insulin resistance and transient hyperglycemia or "glucose tolerance test diabetes" in the non-obese siblings and parents which also manifested evidence of some stimulation of growth of islands.

There is no histologic explanation of the cause of insulin resistance in these mice. Experimentally and clinically, apparent insulin resistance can sometimes be correlated with insulin hypersensitivity, acidosis, infection, toxemia, circulatory failure, renal glycosuria, liver disease, hemochromatosis, or pituitary-adrenal hyperfunction.[2,11] The syndrome of centripetal obesity, insulin resistance, diabetes, and skin changes associated with pituitary or adrenal hyperactivity in humans is comparable to the state in the obese mice. No pituitary or adrenal pathologic changes have yet been found in these mice to support such a possibility, but it has been shown that the insulin antagonistic effect of pituitary extract is mediated in an enzymatic, metabolic reaction.[4] Possibly insulin resistance in these animals is associated with such an enzymatic effect.

Association of abnormalities of fat distribution with peculiarities in glycogen deposition is indicated. The low levels of liver glycogen of obese animals are contrasted with the high levels in non-obese siblings and parents. The hyperglycemia and lability of blood sugar levels of the obese mice are quite different from the stability of blood sugar of non-obese siblings. According to the glycostatic theory of the regulation of appetite, the metabolic defect of the obese animals may be such that they store less liver glycogen, are less able to maintain stable blood glucose levels, and, hence, have great appetites.[12] Whether the resultant excess food intake causes damage to pancreatic beta cells, as Wilder[13] suggested, and whether diabetes occurs as a consequent effect in the obese animals, cannot be determined from the present study. The hypothesis that inability to store liver glycogen increases the appetite in these animals does not, however, explain the peculiar distribution of fat in the obese mice. Although many of the defects in these mice resemble the results of pituitary-adrenal hyperfunction, the low levels of liver glycogen in obese mice are at variance with the

effect of pituitary extracts and corticosterones, which increase gluconeogenesis and promote the deposition of liver glycogen.[14,15] The apparent inability of the obese animals to store liver glycogen may be part of an "inborn error of metabolism" which in turn is not necessarily an endocrine abnormality.

The slow rate of healing of the skin ulcers, some of which were of weeks' duration, may be related to an unfavorable metabolic milieu for tissue growth. Conceivably, of course, it could be related to pituitary or adrenal hyperactivity.[16] The skin ulcers, on smear and culture, revealed enterococci, alpha streptococci, and proteus bacilli, all skin contaminants. The cocci seen microscopically in the ulcer site probably did not cause the skin breakdown, for there was little exudation or inflammatory reaction, and the organisms were scarce at the margin of ulceration but numerous in the centers. Occlusion of vessels was not demonstrated, and there was no evidence of neurogenic atrophy of skin or underlying tissues. These skin changes resemble the "dermatitis gangrenosa" described by Riven[17] in a human patient in diabetic coma. The ulcerations he described arose at sites of pressure: the flank, posterior portions of thighs, and legs. The consistent gluteal or shoulder localization of the ulcers in the mice, at the regions of marked fat accumulation and of pressure during squatting or feeding, indicates a decubital factor in the causation of these ulcerations.

Complications of diabetes were rare in these animals. None of the mice became comatose and no acetone or diacetic acid could be detected in the urine of obese mice; but infection, trauma, change or marked restriction of diet, and physiologic hardships such as pregnancy were avoided. A few obese, diabetic animals did develop fatty livers. The absence of glycogen nephrosis seems surprising in consideration of the large amounts of glucose excreted in the urine of the obese animals.[18] However, the blood sugar level at which glycogen nephrosis develops in mice is not known. Arteriosclerosis, glomerulosclerosis, hepatic cirrhosis, xanthomata, and fatty macrophage accumulation in the spleen were not found; however, none of the animals lived longer than 7 months. The tissues of the obese animal which died at the end of that time were too poorly preserved for accurate diagnosis.

It is hoped that further studies will better indicate the cause of the abnormalities of blood sugar content, of hepatic glycogen distribution, of fat deposition, of pancreatic islet size, and of insulin response. The supply of mice is still limited, but studies of the effect of ACTH administration, alloxan therapy, ageing, and endocrine organ extirpations are being pursued. A number of physiologic, chemical, and histochemical studies are indicated.

Summary

A strain of obese mice with constantly associated diabetes and insulin resistance has been studied in comparison with clinically normal siblings and parents, and Swiss mice. The obesity is inherited as a simple mendelian recessive.

The obese mice exhibit larger, more numerous islands of Langerhans and other evidences of a stimulus to island growth. The siblings manifest slight evidence of a similar stimulus. Structural changes of other endocrine organs have not yet been demonstrated. The obese mice have less than normal amounts of hepatic glycogen. Cutaneous atrophy and ulcers which did not heal were found in some of the obese animals.

The association of changes in the islands of Langerhans with insulin resistance and hyperglycemia suggests the existence of an insulin antagonistic factor which results in island growth primarily or secondarily. This may be an inherent metabolic antagonist.

The low liver glycogen content in obese mice and the lability of blood sugar levels implicates the glycostatic theory of the regulation of appetite in the causation of the obesity, and possibly of the diabetes, in these experimental animals.

We wish to express our sincere appreciation to Mr. Tom Faherty and Miss Margaret Bates for technical assistance, to Mr. Leo Goodman for photographic aid, and to Drs. Stanley Robbins and Henry Jackson for encouragement and advice.

ADDENDUM

Subsequent studies by Guggenheim, Mayer, and Dickie (in preparation) indicate that obese animals do not oxidize administered radioacetate to the same extent as non-obese animals. Other studies by Mayer, Russell, Bates, and Dickie (in preparation) show that the diabetes occurs at the ninth week, is precipitated by growth hormone administration, and that the blood sugar of obese animals is strikingly increased by growth hormone, while that of non-obese animals is not affected.

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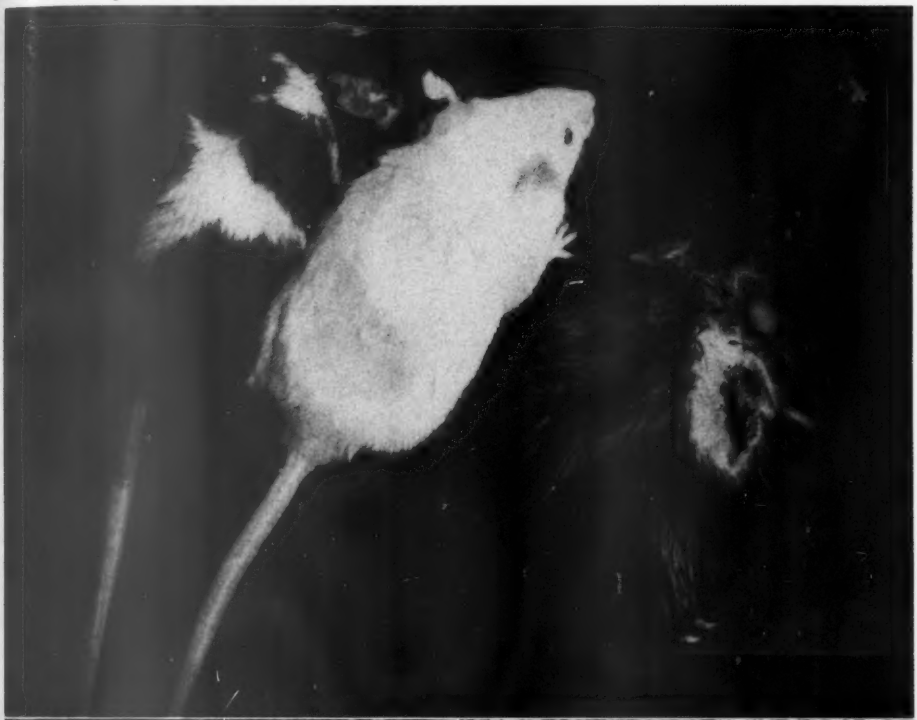
DESCRIPTION OF PLATES

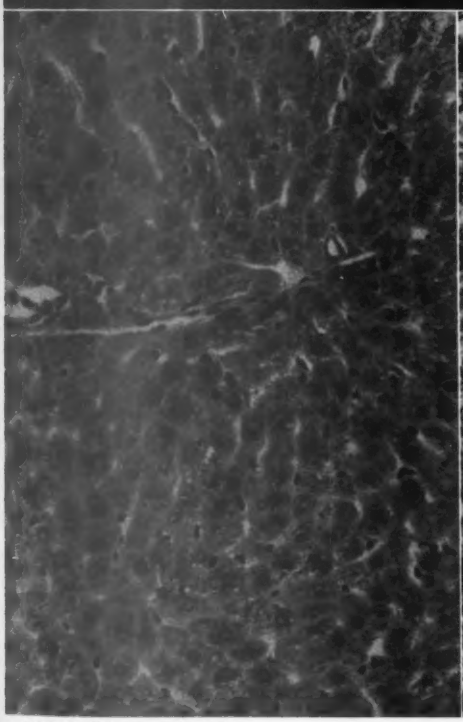
PLATE 60

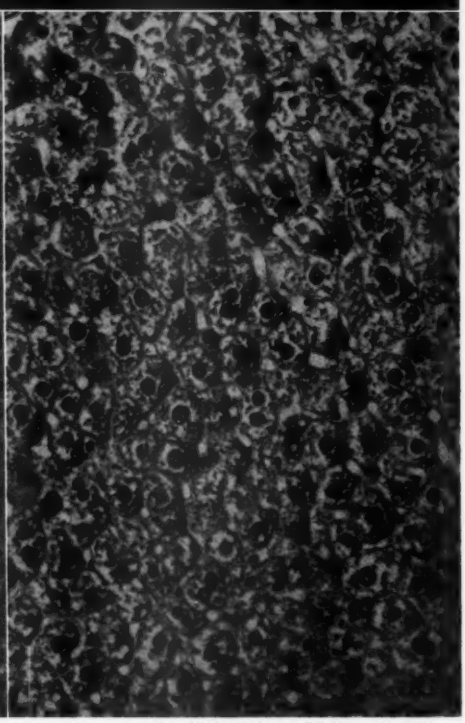
FIG. 1. Obese mouse (gray), non-obese sibling (black and white), and normal control (white). The obese animal has large ulcers of the right shoulder and of the squatting surface of the trunk (not shown).

FIG. 2. From a section of liver of an obese, diabetic, insulin-resistant mouse stained with Best's carmine stain for glycogen. Of note are the small number of granules of stained glycogen. × 180.

FIG. 3. Liver of non-obese, non-diabetic sibling, stained with Best's carmine for glycogen. There are many granules of stained glycogen. × 180.







Bleisch, Mayer, and Dickie Familial Diabetes Mellitus in Mice

PLATE 61

FIG. 4. Liver of control Swiss mouse stained for glycogen. Moderate numbers of granules of carmine-stained glycogen may be seen. × 180.

FIG. 5. Pancreas of obese, diabetic, insulin-resistant mouse. The islets are large, numerous, and composed of cells arranged in a distinctive trabecular pattern. Hematoxylin and eosin stain. × 80.

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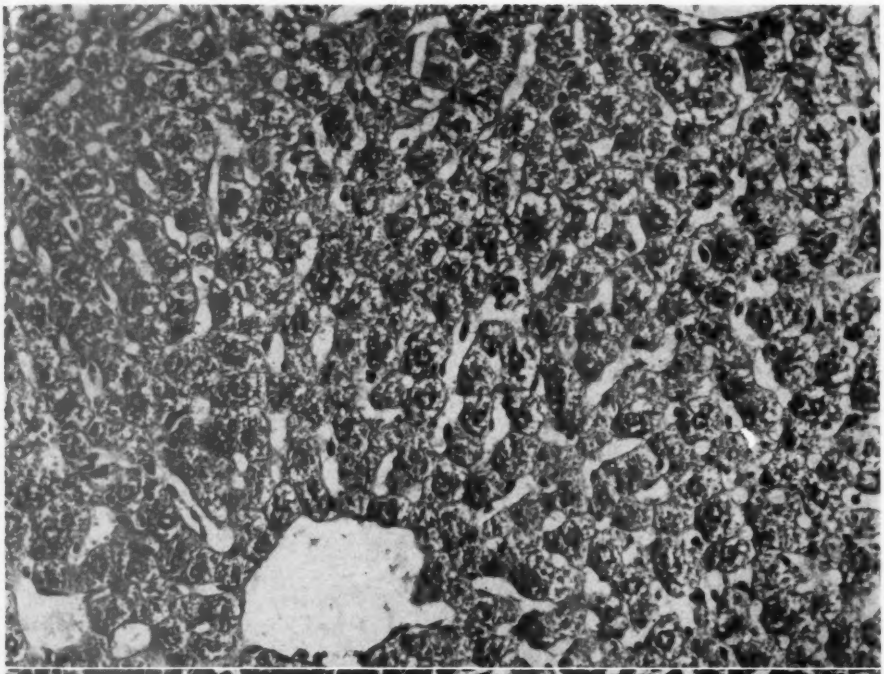

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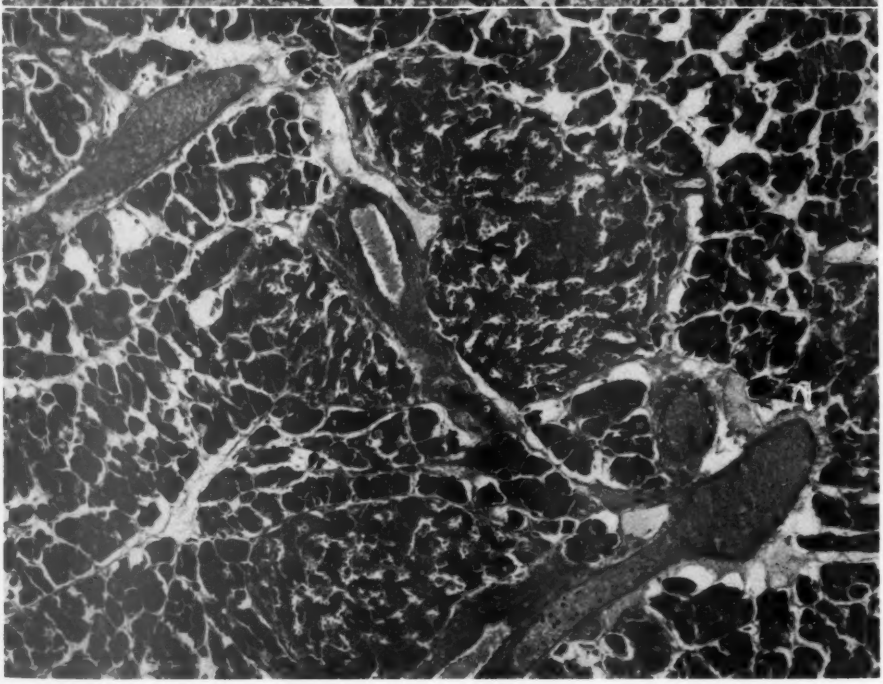
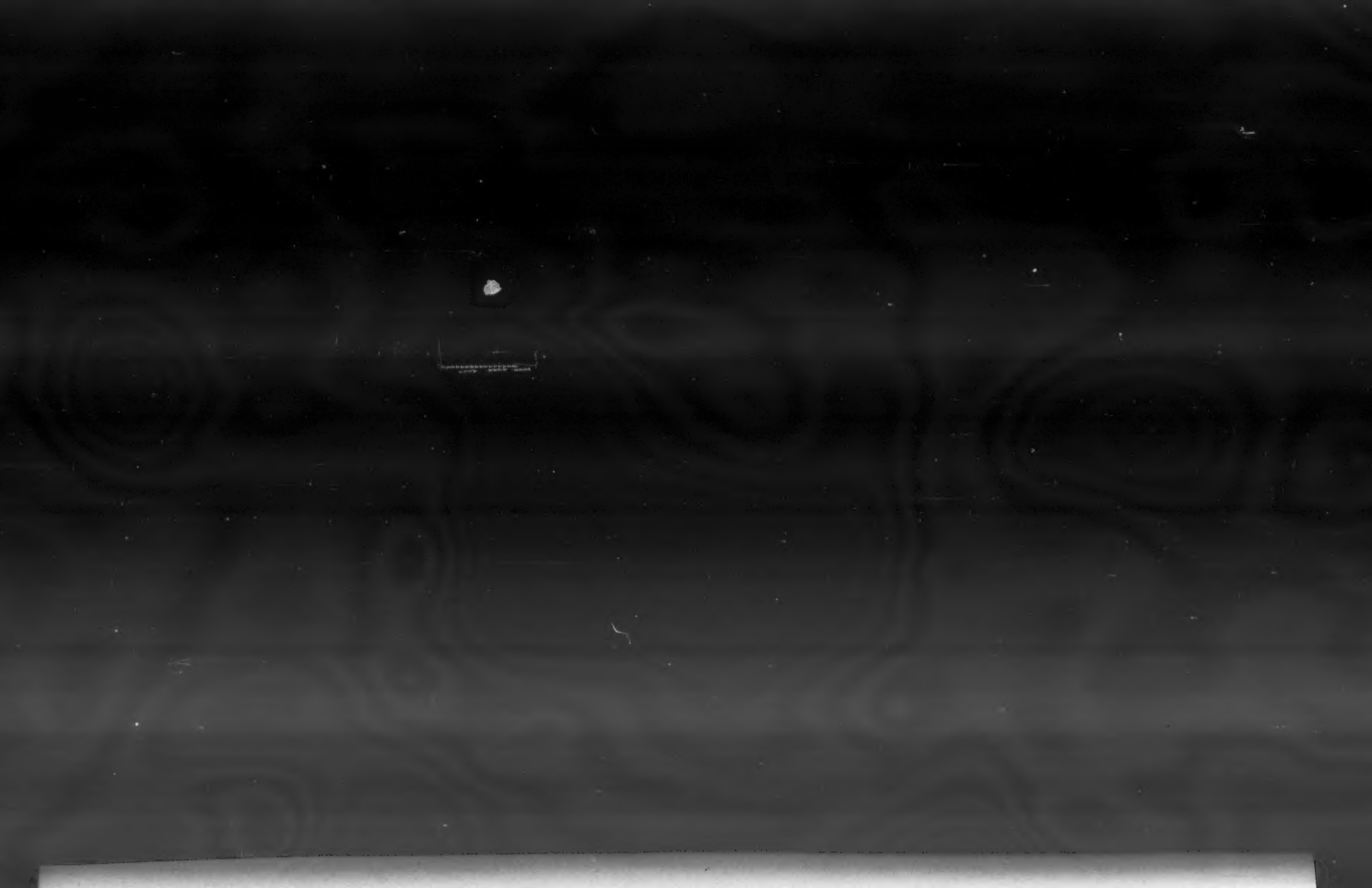


Bleisch, Mayer, and Dickie Familial Diabetes Mellitus in Mice

PLATE 62

FIG. 6. Pancreas of non-obese, non-diabetic sibling. The islands of Langerhans are large and numerous. Hematoxylin and eosin stain. × 80.

FIG. 7. Pancreas from control normal Swiss mouse. The two small islets are characteristic of the normal animals. Hematoxylin and eosin stain. × 80.



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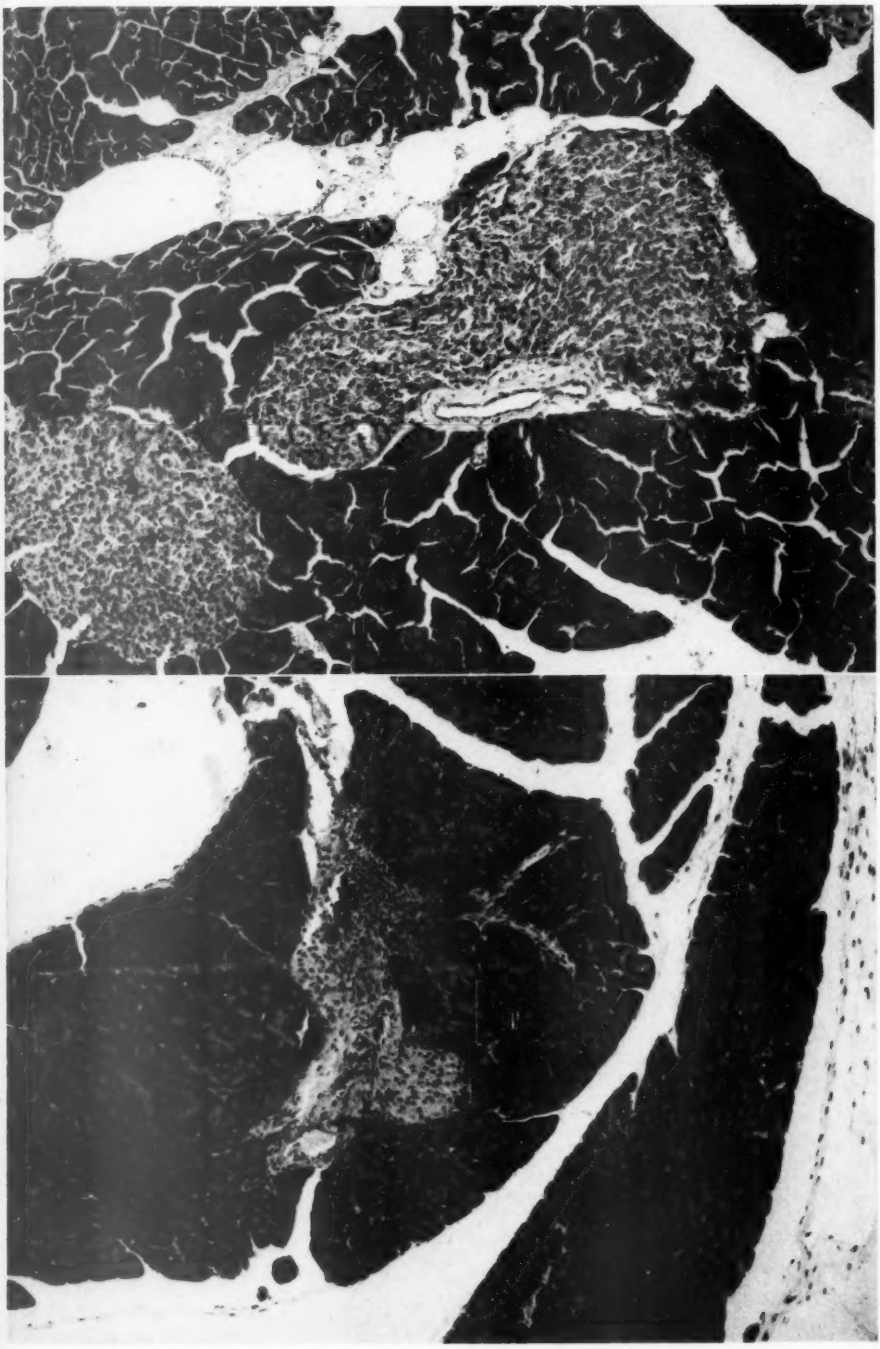

THE DISSOLUTION OF FATTY CYSTS IN PRECIRRHOTIC AND CIRRHOTIC LIVERS OF CHOLINE-DEFICIENT RATS TREATED WITH LIPOTROPIC FACTORS *

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Much of the stainable lipid in livers which have become cirrhotic following prolonged and excessive accumulation of fat is not contained within single parenchymal cells but is enclosed by a number of cells (up to sixty) which together form pathologic fatty cysts. In paraffin sections the cysts are represented by spaces which may attain diameters five or six times that of a normal liver cell (comparative volumes would differ by more than one hundred times). The evidence for the existence of these cysts and a description of their pathogenesis have been published previously.[1-4] The cysts are concentrated in those regions of the hepatic lobule in which trabeculation develops in the cirrhotic liver. This arrangement is not merely a matter of chance. It has been shown that in cirrhosis associated with deficiencies of choline and its precursors, the trabeculation is largely the result of condensation of those reticulin fibers which supported the cells of the cyst walls. These cells (and the cysts) have atrophied and disappeared. As a part of this sequence the fat in the cysts leaves the liver.[3] This is the reflection, on a microscopic level, of the gross observation that the extents of cirrhosis and steatosis are frequently inversely related.[5]

The fat may leave the liver under these conditions by either the biliary or vascular systems; the evidence for this has been presented elsewhere.[3] Restoration of lipotropic factors to the diet of experimental animals at any stage before or during the development of cirrhosis produces a striking fall in the level of hepatic lipids.[6,7] In the cirrhotic liver much of the fat is within cysts. Does the fat leave the cysts of such animals during treatment with choline by the biliary or vascular routes, or are other mechanisms invoked? The data in this paper show that under the influence of choline some of the intracystic fat returns to the normal liver cells where it presumably is metabolized.

Methods

Fifty Wistar albino rats (both male and female) which had received a hypolipotropic diet[3] for 8 to 12 months, were maintained on this same basal food-mixture with an added choline supplement (0.35 per

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cent choline chloride) for an additional period ranging from 3 days to 3 months. The animals were sacrificed under ether anesthesia and multiple blocks of liver tissue were fixed for 6 to 24 hours in Bouin's solution for paraffin sectioning, or in Baker's formol-calcium[8] for frozen sectioning. To reduce traumatic artefact to a minimum, the frozen sections were floated on a solution of 10 per cent formol-saline and transferred immediately to gelatinized glass slides, to which they were affixed by exposure to formalin vapor for at least 30 minutes. Thus at all stages of the staining procedure (Wilson technic[9]) the sections were adequately supported and the introduction of manipulative artefact was reduced to a minimum. Hematoxylin and eosin stains were used on all Bouin-fixed paraffin sections, and an assortment of special stains were applied to selected sections to demonstrate connective tissue, ceroid pigment, and iron deposits.

The Cytology of Fatty Cysts

Before proceeding to a description of the alterations in cysts after choline has been restored to the animals' diet, it is necessary to consider the cellular structure of the cyst wall in the choline-deficient rat. The lumen of a cyst is filled with fat and its wall is made up of a variable number (two to sixty) of simple epithelial cells. These are derived from parenchymal cells which have become greatly modified during the formation of the cyst. These cells, although simple, are adapted to their function in a highly interesting manner.

The Cyst Wall in Cross Section

When viewed on edge (Fig. 1), as they most frequently are in random sections, the cells in the wall of a cyst appear greatly flattened and even atrophic. The cytoplasm extends in both directions from the nucleus, as a thin, curving plate which joins that of neighboring cells in the wall. The junctions between cells are often difficult to see in these "edgewise" views. The nucleus appears to have stained so darkly that pyknosis is suggested (Fig. 1); but as will be seen when considering tangential sections of cysts, this may often be misleading. Similarly, the cytoplasm which in cross section appears almost devoid of internal structure, will be shown to contain the usual inclusions and organelles associated with functioning parenchyma.

The Cyst Wall in Tangential Section

When the microtome knife passes through the edge of a cyst tangentially, the two-dimensional picture in the resulting section differs strikingly from that seen in the more conventional cross-sectional view.

Tangential sections afford the observer an opportunity to study the cells of the cyst wall *en face*. It is then apparent (Figs. 2, 3, and 4) that these cells are compressed only in one dimension and that this is an adaptation in structure to meet their function of retaining fat within the cyst lumen. The nucleus, which on edge appeared rod-like and dark, *en face* is large and round (Fig. 2), and within its membrane may be easily discerned a large nucleolus, nucleolar satellites, and a network of chromatin. This indicates that neither is the cell atrophic nor the nucleus pyknotic. The cytoplasm in similar sections can be seen to surround the nucleus and extend out in every direction so that it resembles a disk. The junctions between neighboring cells may be easily visualized and these resemble the junctions between cells in any flattened type of epithelium. Within the cytoplasm, mitochondrial rodlets and other organelles may be visualized with the aid of suitable stains or phase microscopy. Stainable fat *within* the cytoplasm of the cells of cysts in choline-deficient rats is minimal or absent, although in frozen sections fat completely or nearly completely fills the lumen of most of the cysts (Fig. 11). These observations indicate that although these cells are specially adapted to form an epithelial lining which is remarkable for being only 1 or 2 μ in thickness, this is not necessarily associated with any regressive or atrophic change. This point is of importance for an understanding of the series of changes which occur in mural cells when choline is restored to the animals' diet. These will now be described.

Changes in Cysts When Choline Is Restored to the Diet

The alterations in cysts now to be considered are discussed under three headings (stainable fat in the walls of cysts, escape of lipid from cysts, and the final dissolution of cysts), but this is merely to facilitate description. Actually, all stages may frequently be seen in a single liver section and sometimes even in different regions of the same cyst.

Reappearance of Stainable Fat in the Cyst Wall

The cytoplasm of cells involved in cyst formation is initially laden with fat. When adjacent cells conjoin to form cysts, this intracellular fat escapes from within the cytoplasm due to rupture of the cell membrane. As noted earlier, little or no stainable fat is then present within the cytoplasm of the flattened mural cells. When choline is restored to the animals' diet, the first visible result is seen in frozen sections of the cysts, for stainable fat now appears within the cytoplasm (Figs. 5 and 11). This may be seen as early as 3 days after the animals have been placed on the supplemented diet. The cytoplasmic plates may not

be appreciably thicker than in the period of choline deficiency, but many small droplets of stainable fat are present throughout. This may give the entire cell a red hue so that it stands out clearly defined from nearby parenchymal cells which are free of visible fat (Figs. 5 and 6). This indicates that alterations of a special nature have been produced in the mural cells by the restoration of dietary choline. Essentially, this consists of a reversal of the phenomenon which occurs in the choline-deficient rat during formation of cysts; *i.e.*, the conversion of intracellular to extracellular fat. There is no proof that the fat which appears in the mural cells under the influence of choline is derived from that in the lumen of the cyst, but all morphologic evidence strongly suggests this. Proof could be obtained only by special investigations in which labelled fat was employed.

An interesting finding concomitant with the appearance of fat within mural cells is the frequent absence of stainable fat within the lumina of the corresponding cysts as seen in thin (4 or 5 μ) frozen sections. This is the exception, rather than the rule, in livers of choline-deficient animals. Either all the fat has left the cyst lumen after treatment of the animal with choline for only a few days or, more likely, the fat is held less firmly within the cyst and is readily lost during the sectioning and staining procedures. This is additional evidence to suggest that the intracellular fat in the mural cells of the cyst is derived from the lumen of the cyst.

In most instances, mural cells which contain visible fat possess a more rounded or hexagonal shape (even when sectioned tangentially) than those which are free of stainable lipid (Fig. 6). The fatty cells have lost their special, flattened shape. This may be due to a swelling effect associated with the entry of the fat into the cytoplasm. In any event, this leads to retraction and dissociation of adjoining cells (Fig. 7), so that their junctions are now more prominent and this may facilitate emptying of the cyst by more direct routes, to be considered later in this study.

It may well be asked how fat within a cyst lumen may pass through the limiting membranes of mural cells when choline is available. A process of hydrolysis or phosphorylation may be suggested. The problem is akin to that of how fat passes through the cell membrane of the epithelium covering an intestinal villus.

Escape of Cyst Fat Through Biliary and Vascular Channels

In choline-deficient rats it has been established[8] that, following rupture, the fat in cysts may be removed through either biliary or vascular channels. Examination of microsections of livers of animals treated

with choline indicates that these pathways are still employed, in addition to the resorptive mechanism described in the preceding paragraphs. The conversion of mural cells from thin plates to a more normal polyhedral configuration seems to be accompanied by widening of the interstices between the cells in the wall (Fig. 7). Such interstices often may be continuous with the lumina of either canaliculi or sinusoids, as has previously been demonstrated.[3] Thus the escape of fat from the cyst lumen into neighboring portions of the biliary or vascular systems is facilitated.

It is difficult to assess the relative importance of the resorptive process in relation to the routes considered here. It would be of interest to know by which of these alternative pathways the greatest amount of fat is removed. Examination of sections from all animals revealed that in an individual cyst any one mechanism may predominate, but taken *in toto* it has been impossible to ascribe greater importance to a particular method.

Eventual Dissolution of Cysts

No matter how the lipid departs from the cysts, as soon as this process has neared completion the structure of the cysts begins to dissolve (Figs. 11 and 12). The rosette disposition of the mural cells persists for some time, but they no longer form a continuous lining membrane. The cells regain their individual character and revert to the characteristics of normal parenchyma. At this stage, when cut in any plane, they are rounded or polyhedral with centrally placed, spherical nuclei. This process is accompanied by diminution in the diameter of the lumen of the cyst, which is encroached upon by the surrounding cells. By these stages, each cell withdraws from its neighbors, takes up space from the lumen, and becomes lost in the cords or plates of hepatic parenchyma.

Occasionally, a variant in this sequence of events is encountered. The lumen of the cyst has become small, but the surrounding mural cells have not reverted to the appearance of normal liver cells (Figs. 9 and 10). Instead they have become crowded together around the contracted lumen so that many are included in single random sections. Were it not that all gradations in form can be observed (Fig. 9), one would not be likely to associate these cells with the usual mural cell. The nuclei are dark, pyknotic, and assume bizarre forms. The cytoplasm is scanty and stains more intensely than before. With the lower magnifications, these could easily be mistaken for small, round cells, but higher magnification reveals that they differ significantly from lymphocytes in respect to their nuclear shape and nuclear-cytoplasmic

ratio. It is possible that these represent mural cells which have undergone such extensive differentiation that they are unable to revert to the normal state when choline is restored to the diet.

Identification of Cysts and Their Various Stages in Routine Sections

There is considerable evidence that certain types of fatty cirrhosis in man, particularly that associated with excessive consumption of alcohol, are related to the cirrhosis in rats fed low-choline diets.[10] From our experimental observations it is apparent that the microscopic appearance of cysts in sections may afford the practical pathologist an indication whether the cirrhosis is progressing or regressing. It is therefore important to define the criteria by which cysts may be identified and their microscopic features interpreted.

The Identification of Cysts in Sections

It is not always apparent whether large fat vacuoles are contained within single cells or lie in small cysts. The limiting membranes between two adjoining cells each distended by a large spherule of fat may fuse into a single morphologic septum which becomes stretched and tenuous and is eventually ruptured. This results in the formation of a central pool of lipid which is now surrounded by the two conjoined parent cells. By definition,[1] this structure is now a small, two-celled, fatty cyst. Unless the microtome knife has passed through the greatest diameter of such a microcyst, it would be impossible from a single random section to distinguish it from a large intracellular fat vacuole. It is apparent, however, that the larger a cyst, the greater will be the number of cells in its wall. Identification of cysts thus becomes progressively easier as they become larger, for the number of mural nuclei in single sections becomes greater. These are the characteristics which are the most useful for the identification of cysts in routine paraffin sections.

Identification of Stages of Regression in Cysts

The cardinal feature of cysts which are regressing and disappearing is the presence of appreciable amounts of stainable fat within the cytoplasm of the cells forming their walls. This serves to emphasize the importance of utilizing frozen sections stained for fat in examining material taken from livers of this type. In paraffin sections, vacuolation of the cytoplasm of the mural cells can sometimes be discerned, but unless it is known that the vacuoles contain lipid, this finding cannot be relied upon as an index for resorption of fat contained within

cysts. The best indication that can be found in paraffin sections to suggest that fatty and cirrhotic lesions are arrested, is the presence of parenchymal cells arranged in rosettes, as shown in the cyst in Figure 12. Such configurations afford definite evidence that cysts are breaking up and their constituent cells reverting to a normal parenchymal pattern. It is suggested that these features, when present in specimens for biopsy from the fatty or cirrhotic livers of chronic alcoholic patients, could be interpreted as strong presumptive evidence that the progress of the pathologic lesions has been arrested.

Conclusions

Treatment of choline-deficient precirrhotic or cirrhotic rats with lipotropic factors for periods up to 3 months brings about dissolution and eventual disappearance of nearly all pathologic fatty cysts encountered in this condition.

After choline is restored to the diet of such animals, intracellular lipid can be demonstrated within the cytoplasm in the walls of the fatty cysts. This is associated with a decrease in the lipid found within the lumina of the cysts.

Fat may also leave the cysts through the biliary or vascular route in a manner similar to that previously found to occur in choline-deficient rats not treated with lipotropic agents.

After the lumina of fatty cysts have been emptied by any of the mechanisms described, the cells in the cyst wall re-assume the appearance of normal parenchyma and, after passing through a stage in which they exhibit rosettes, re-form in the usual cord or plate pattern. This is associated with an encroachment on the lumen of the cyst so that it is eventually obliterated. An abnormal variant of this procedure may occur in which the cytoplasm is scanty and the nuclei pyknotic.

If cysts are observed in any of the stages of disappearance, this may indicate that the fatty and cirrhotic lesions are actually regressing.

We are indebted to Professor C. H. Best for his interest and stimulation throughout the course of these investigations. Grateful acknowledgment is made to Dr. Jessie H. Ridout for her invaluable assistance in the preparations of diets and in the care of the animals. Messrs. William Wilson and Overdo George prepared the histologic material and Mrs. Kathleen Henderson assisted with the photographic material.

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DESCRIPTION OF PLATES

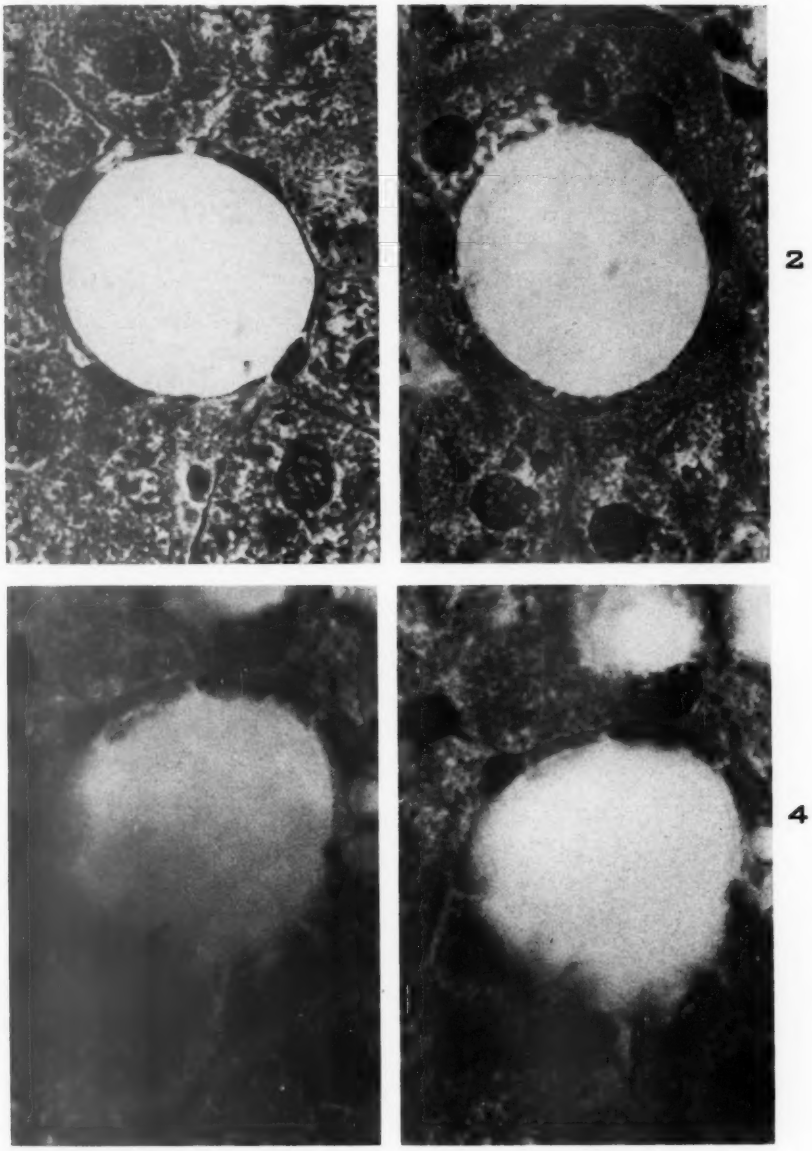
All photomicrographs are of livers of choline-deficient rats treated with lipotropic factors for varying periods of time (with the exception of Fig. 1). Magnifications throughout are × 800, unless otherwise stated.

PLATE 63

FIG. 1. A cross section of a cyst, which fills the center of the field, has included four mural cells which are seen on edge. Of note are the thinness of their cytoplasmic plates and the basophilia of the nuclei. Paraffin section, hematoxylin and eosin stain.

FIG. 2. The plane in which this cyst has been sectioned is tangential to its greatest circumference. The four nuclei at the top of the field are seen *en face*. Within the nuclei, nucleoli and a chromatin network are clearly visible. The cytoplasm is distributed almost equally around the nuclei. If these cells had been sectioned in a plane comparable to that in which the mural cells of Figure 1 were cut, their appearance would have been similar.

FIGS. 3 and 4. The same tangential section of a fatty cyst is seen in each photomicrograph. At the upper pole, the nucleus (in cross section) is thin and basophilic. The nuclei at the lower pole are seen almost full-face, and are surrounded with cytoplasm. The focus in Figure 4 is deeper than in Figure 3, and greater roundness (cupping) of the nuclei is apparent.



Hartroft and Sellers Dissolution of Fatty Cysts of the Liver

PLATE 64

FIG. 5. A cross section of a fatty cyst 8 days after restoration of choline to the diet. The intense staining of the cytoplasm in the mural cells is due to myriads of fat droplets. Of note also are the thickening of these cells and the absence of stainable fat within the lumen of the cyst. Orange red O stain.

FIG. 6. A cross section after 12 days, a later stage of the same process. The mural cells (outlined by dark-staining fat droplets) have regained the characteristics of parenchymal cells. Little or no fat remains in the lumen. Orange red O stain.

FIG. 7. The mural cells again resemble parenchymal cells. Although they contain fat, they have become dissociated in the wall of the cyst and canaliculi can be seen between them. Orange red O stain.

FIG. 8. Fat is seen in the sinusoids in proximity to, and in connection with, a fatty cyst at the right. Orange red O stain.

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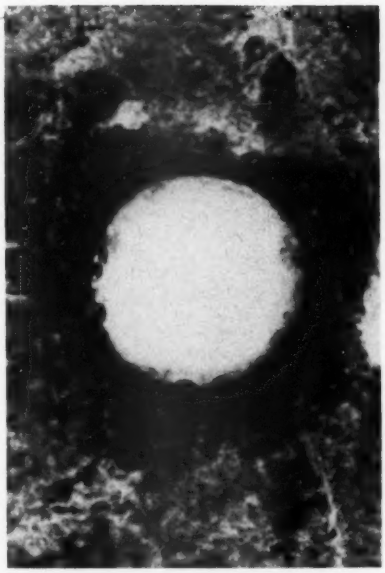

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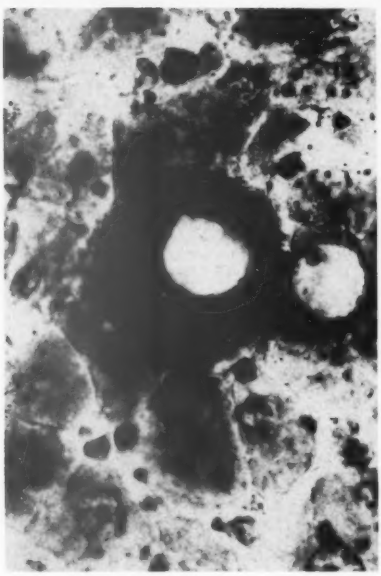

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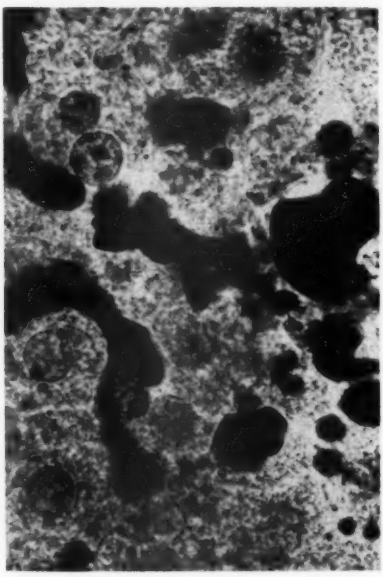

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Hartroft and Sellers

Dissolution of Fatty Cysts of the Liver

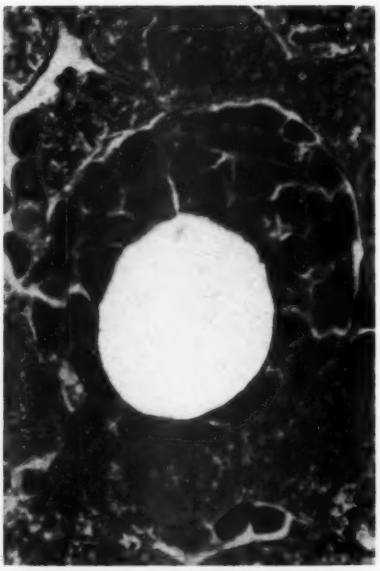
PLATE 65

FIGS. 9 and 10. Collections of abnormal mural cells occasionally seen as a variant in the later stages of the process of dissolution. In both figures some cells resemble the parenchymal type while numerous transitional forms, many of which show signs of cellular degeneration, are present also.

FIG. 11. Cross section of a fatty cyst. Fat from the cyst lumen is bulging into the interstices between mural cells. The cytoplasm of these cells contains fat droplets, and is distributed around nuclei which appear larger and more nearly circular than previously (*cf.* Fig. 1). Orange red O stain. × 1000.

FIG. 12. A rosette of large, round nuclei, each surrounded with cytoplasm containing fat droplets, encroaches upon a clear area which apparently is all that remains of a fatty cyst. Orange red O stain.

9



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Hartroft and Sellers Dissolution of Fatty Cysts of the Liver

ENDOPHLEBOHYPERTROPHY AND PHLEBOSCLEROSIS

II. The External and Common Iliac Veins*

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In a previous report,[1] the processes of endophlebohypertrophy and endophlebosclerosis were described in the popliteal vein. The former process occurs from birth at the mouths of tributaries and adjacent to the popliteal artery. It is typified grossly by longitudinal streaks and plaques. Histologically, the plaques result from proliferation in a longitudinal direction of elastic, muscle, and connective tissue of the intima and the intimal aspect of the media, lying in an increased amount of ground substance. These plaques, grossly, undergo changes with advancing age, becoming broader, deeper, firmer, and more geographic. Correspondingly, retrogressive changes are found histologically which are included under the term endophlebosclerosis. These changes are: (1) basophilic staining, vacuolization, and lacunar formation of the ground substance, (2) disruption and loss of elastic fibers, (3) loss of muscle cells, (4) fragmentation of the internal elastic lamella, (5) increased and decreased staining of the glycoprotein and reticular components of the basement membranes around muscle and elastic fibers of the intima, and (6) replacement by fibrous connective tissue. Endophlebosclerosis was differentiated from senile sclerosis, which is typified by a diffuse increase in connective tissue and focal calcification of the intima.

The present report deals with similar changes in the external and common iliac veins. The historic aspect of this subject has been dealt with previously.[1] The pattern of study followed in our present work is identical with that employed for the popliteal vein. A study was made of (1) the functional gross anatomy of the vein, (2) the gross architecture of the vein and its changes with advancing age (excluding cases with increased venous pressure), (3) the microscopic architecture of the vein and its changes with advancing age (excluding cases with increased venous pressure), and (4) a comparison of the gross and microscopic architecture of the vein, with and without increased venous pressure.

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‡ The Department of Pathology of Michael Reese Hospital is in part supported by the Michael Reese Research Foundation.

Material and Methods

The external and common iliac veins in routinely necropsied patients were dissected in their entirety from beneath the inguinal ligaments to the bifurcation of the inferior vena cava. One hundred and sixty-two veins were thus obtained from patients of various ages (Table I). The

Table I

External and Common Iliac Veins Studied According to Age Groups

Age groups	Number	Age groups	Number
yrs.		*yrs.*	
0–10	21	51–60	21
11–20	6	61–70	42
21–30	7	71–80	24
31–40	19	81–90	6
41–50	16	All ages	162

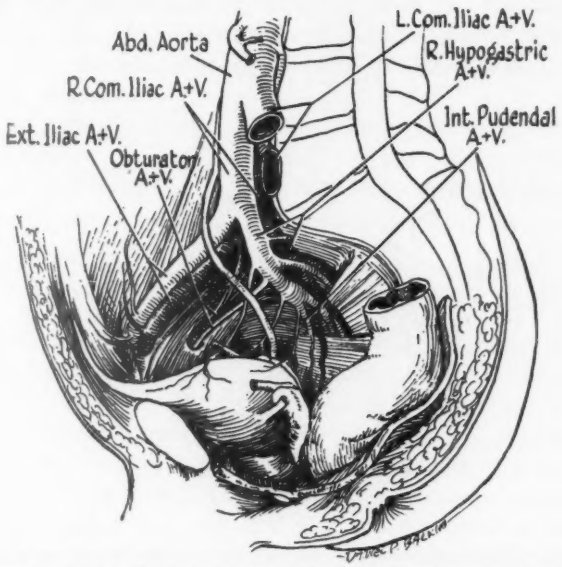
veins were opened immediately following removal, and their gross architecture was carefully noted. They were then fixed in 4 per cent formaldehyde solution, and numerous sections were taken in the longitudinal and transverse planes. They were embedded in paraffin, sectioned, and stained with hematoxylin and eosin, and Weigert's elastica counterstained with van Gieson's stain. Some sections were stained with orcein, Heidenhain's azan, Masson's trichrome, the McManus-Lillie-Hotchkiss glycoprotein stain for ground substance and basement membranes, Gomori's stain for reticulum, Lillie's modification of Langeron's alizarin red stain for calcium, and Sudan IV for fat. In addition, portions of vein were prepared by freezing and drying by the Altmann-Gersh technic and embedded in paraffin. Sections were then stained with hematoxylin and eosin, and for glycoprotein. Other sections were kept in 95 per cent alcohol overnight before staining for glycoprotein.

Functional Gross Anatomy and Basic Histology

The external iliac veins commence at the distal border of the inguinal ligament, being the extension of the femoral veins (Text-figs. 1 and 2). In their ascent they lie anterior to the superior rami of the pubic bones and the psoas major muscles. At their termination, where they join the hypogastric veins, they are anterior to the sacro-iliac articulations. They are in intimate contact with the external iliac arteries. This relationship, however, differs on the two sides. On the left side, the vein is medial to the artery. On the right, it is medial below, but in its ascent it gradually comes to lie posterior to the artery.

The external iliac veins receive the inferior epigastric and the deep circumflex veins.

The common iliac veins commence at the sacro-iliac articulations, and converge to form the inferior vena cava at the upper border of the fifth lumbar vertebra. Anatomically, the right and left common iliac veins differ considerably. The right vein is relatively short and more vertical in direction than the left. At its origin, the right vein lies be-



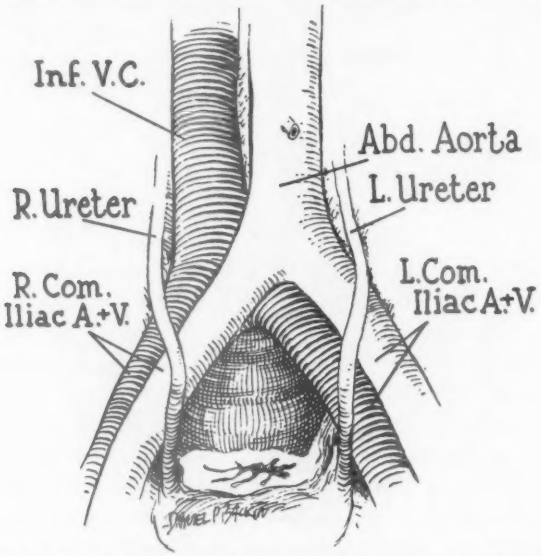
Text-figure 1. Form and relations of the right external and common iliac veins. After Morris and Schaeffer's *Human Anatomy*, The Blakiston Co., Philadelphia.

hind the corresponding artery, but in its upward course it gradually assumes a position lateral and slightly posterior to the artery. It is anterior to the wing of the sacrum and to the lateral part of the body or the lateral process of the fifth lumbar vertebra. The left common ilac vein lies medial and slightly posterior to the artery. At its junction with the left external iliac vein it lies posterior to the left internal iliac artery. In its ascent, it lies anterior to the promontory of the sacrum and the body of the fifth lumbar vertebra, and at its termination it is posterior to the right common iliac artery.

Thus the external and common iliac veins are subject to external stresses of the corresponding arteries. In addition, the termination of the left common iliac vein is subject to pressure from the right common iliac artery. The junction of the left external and common iliac veins bears the stress of the left internal iliac artery. Likewise, the

upper margins of the superior rami of the pubic bones and psoas major muscles exert stress on the external iliac veins, the fifth lumbar vertebra and the wing of the sacrum on the right common iliac vein, and the promontory of the sacrum and the fifth lumbar vertebra on the left common iliac vein.

The left common iliac vein usually receives the middle sacral. Sometimes the common iliac veins drain the iliolumbar vein.



Text-figure 2. Form and relations of the common iliac veins. After Morris and Schaeffer's *Human Anatomy*, The Blakiston Co., Philadelphia.

Certain anatomical deviations in the left common iliac veins were noted in our series. In 4 cases, a septum extended for a varying distance from the mouth of the left common iliac vein into the vessel, dividing its distal part into two channels. In 8 other cases the mouth of the left common iliac vein was narrower than the lumen.

The valvular arrangement in 61 cases in our series, in which this was especially studied, was found to be as follows: A valve was found at the mouth of one right common iliac vein, but no valve was found in the left common iliac vein in any instance. In the external iliac veins, 12 cases presented a valve at the outlet of the right and not of the left, 6 cases at the mouth of the left and not of the right, 14 cases at the mouths of both, and 29 cases presented no valves in the external iliac veins. The junction of the femoral and the external iliac veins always presented a valve on both sides.

The basic histologic characteristics of the external iliac vein are as

follows: The intima consists of an endothelial lining, and an internal elastic lamella. The media consists of several layers of circular muscle, surrounded by fine, longitudinal, elastic fibers and a small amount of collagen. The adventitia is the most extensive layer, consisting of a thick layer of longitudinal muscle (developed after the first year), surrounded by coarse longitudinal or spiral elastic and collagen fibers (Fig. 1). A considerable number of vasa are present in the adventitia and media. The media is thicker in the external iliac vein than in the common iliac vein, but otherwise they are quite similar in structure. Elements of the intima and media are embedded in a small amount of intercellular substance.

From birth to adulthood there is an accentuation of the elastic fibers, and a general increase in thickness of the media and adventitia. In old age, there is an increase in connective tissue throughout the media and adventitia with a relative decrease in muscle cells.

Changes in the Gross and Microscopic Architecture with Advancing Age

Endophlebohypertrophy was found to occur from birth in the external and common iliac veins as in the popliteal. This was seen grossly in the first 2 decades as longitudinal, relatively smooth, white to yellow-white, slightly elevated formations (Fig. 2). These plaques were found to be adjacent to the contiguous arterial tree, as well as adjacent to the contiguous bony and muscle structures as described. Histologically, the process was similar to that described in the popliteal vein. It consisted of a proliferation in the direction of blood flow of elastic, muscle, and collagenous connective tissue in the intima and the intimal aspect of the media, with the basement membranes around the muscle and elastic tissue (Fig. 3). There were, however, some differences worthy of note. The proliferation of elastic fibers was more dominant (Fig. 4) and there was a lesser tendency to produce the layering effect which was seen in the popliteal vein.

As in the popliteal vein, the plaques resulting from endophlebohypertrophy in the iliac veins underwent changes in the third and subsequent decades. They became broader, more yellow, fenestrated, and geographic (Fig. 5). Small calcific craters appeared after 50 years of age. Histologically, the process of endophlebosclerosis as described in the popliteal vein was now seen in these plaques (Figs. 7 to 16). There were, however, some important differences. Fragmentation of the elastic fibers was focally more severe, and destruction and fraying of the internal elastic lamella were common. Correspondingly, the changes in the reticular and glycoprotein components of the basement

membranes around the elastic fibers were more severe (Figs. 13 to 16), and calcium was laid down earlier. Focally present in the intima were neutrophils and lymphoid cells, with degenerated nuclear forms resembling muscle cells as seen in arteriosclerosis (Fig. 17). These changes were not noted in the popliteal vein. In addition, fat stains often revealed small deposits of fat in the intima, less commonly in the intima and media, and least commonly in all layers. This fat was found in the intercellular substance, muscle cells, and along elastic fibers (Fig. 18).* Finally, replacement by connective tissue in the intima was less pronounced than in the popliteal vein.

Rôle of Increased Venous Pressure in Endophlebohypertrophy and Endophlebosclerosis

Thirty cases with definite evidence of increased venous pressure were compared with 73 cases in which there was no such evidence. This comparison was made in each age group (Table II). Increased

Table II

Iliac Veins with and without Increased Venous Pressure According to Age Groups

Age groups	Without increased venous pressure	With increased venous pressure
yrs.		
0–10	19	0
11–20	2	3
21–30	4	1
31–40	10	2
41–50	2	6
51–60	12	2
61–70	16	12
71–80	6	3
81–90	2	1
Totals	73	30

venous pressure was considered to be present when there were both clinical and pathologic evidences of long standing, chronic passive hyperemia of the liver and peripheral edema which were definitely related to a hypertrophied heart. Endophlebohypertrophy and endophlebosclerosis were found to be more diffuse and severe, both grossly and histologically, in cases with increased venous pressure (Fig. 6). In addition, the media was diffusely thicker. There was no demonstrable increase in connective tissue in the adventitia. These findings are

* In our previous paper [1] we stated that fat was not found in endophlebosclerosis of the popliteal vein. Since then an occasional popliteal vein has been found to contain fat.

in contrast to those in the popliteal vein, where no appreciable difference was demonstrated between cases with and without increased pressure.

Interpretation of Results

It was pointed out in our discussion of the popliteal vein that endophlebohypertrophy and endophlebosclerosis are local reactions to mechanical stress. In the popliteal vein, the rôle of the contiguous artery and the entry of tributaries in providing this stress was mentioned. It was considered that the common denominator in their mode of action was a local disturbance in blood flow. In the iliac veins, in addition to these two factors, it is clear that bone and muscle also may act as points of stress.

In the iliac veins we have the further rôle of increased venous pressure. The reaction here is twofold. There is both local increase and greater diffusion of endophlebohypertrophy and sclerosis, and generalized increase in thickness of the media. The first reaction indicates that increased venous pressure produces a more widespread disturbance of blood flow. This occurs not only at points of normal physiologic stress, but elsewhere in the intima as well. At the same time, the increased pressure has a direct lateral effect upon the vein.

The deposition of fat in endophlebosclerosis of the iliac veins is clearly a secondary phenomenon, and most likely related to previous degenerative changes. Endophlebosclerosis is not produced by an alteration in cholesterol or lipid metabolism.

Summary

The processes of endophlebohypertrophy and endophlebosclerosis in the iliac veins are, in general, similar to these processes in the popliteal vein. There are more severe focal degenerative changes in the iliac veins and a tendency to deposit small amounts of fat.

In addition to arterial contiguity and entry of tributaries, bone and muscle act to produce external local stresses on the iliac veins.

Increased venous pressure results in more widespread distribution of endophlebohypertrophy and sclerosis. In addition, there is thickening of the media. It is suggested that this is due to both tangential and lateral hydrodynamic forces.

Acknowledgment is made for the technical assistance of Mrs. Ilsa Schwartz and Mrs. Catherine Malone Bader.

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1. Lev, M., and Saphir, O. Endophlebohypertrophy and phlebosclerosis. I. The popliteal vein. *A. M. A. Arch. Path.*, 1951, **51**, 154–178.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 66

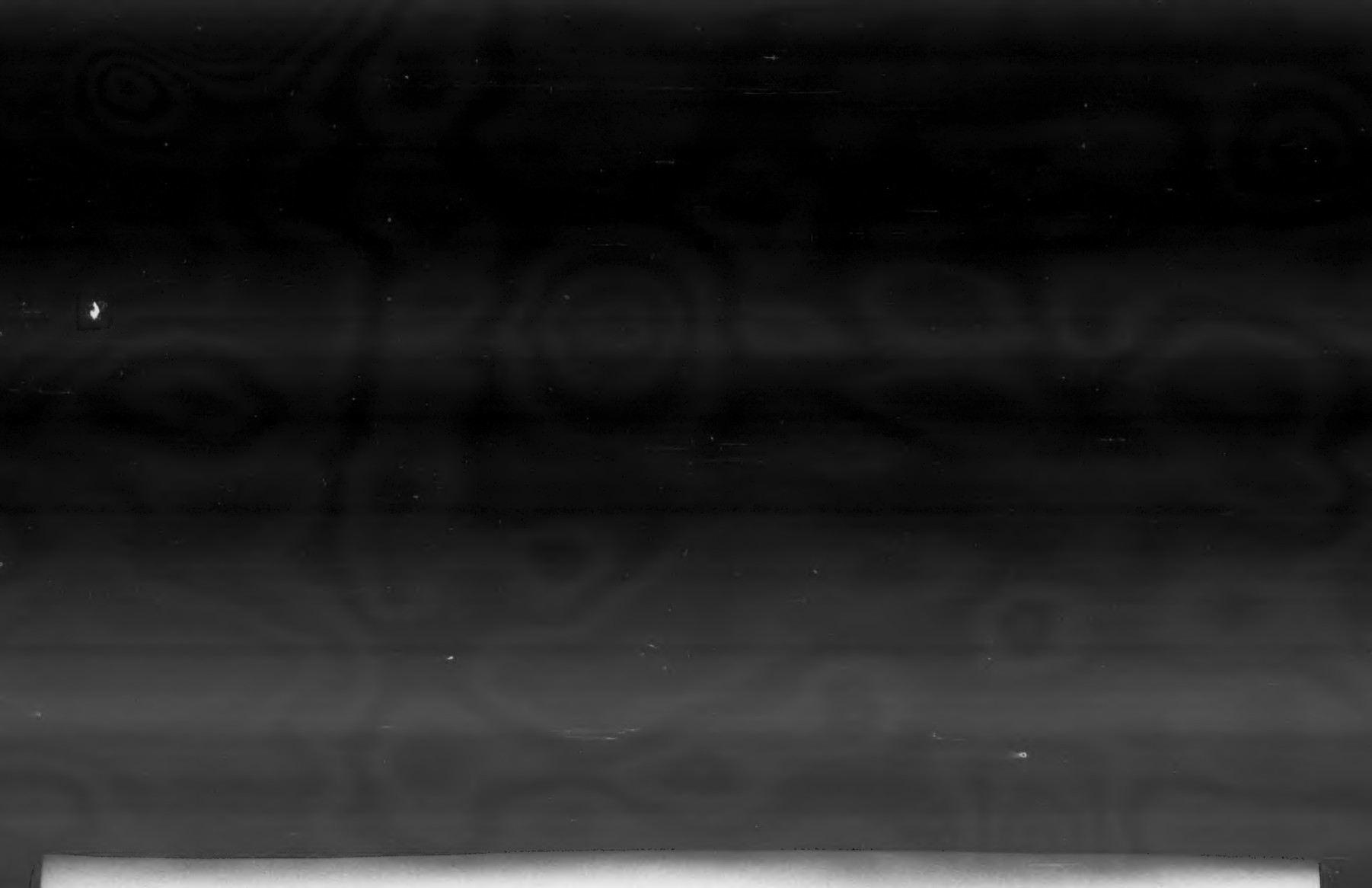
FIG. 1. Basic structure of the external and common iliac veins. Weigert's elastic tissue-van Gieson stain. × 8.4.

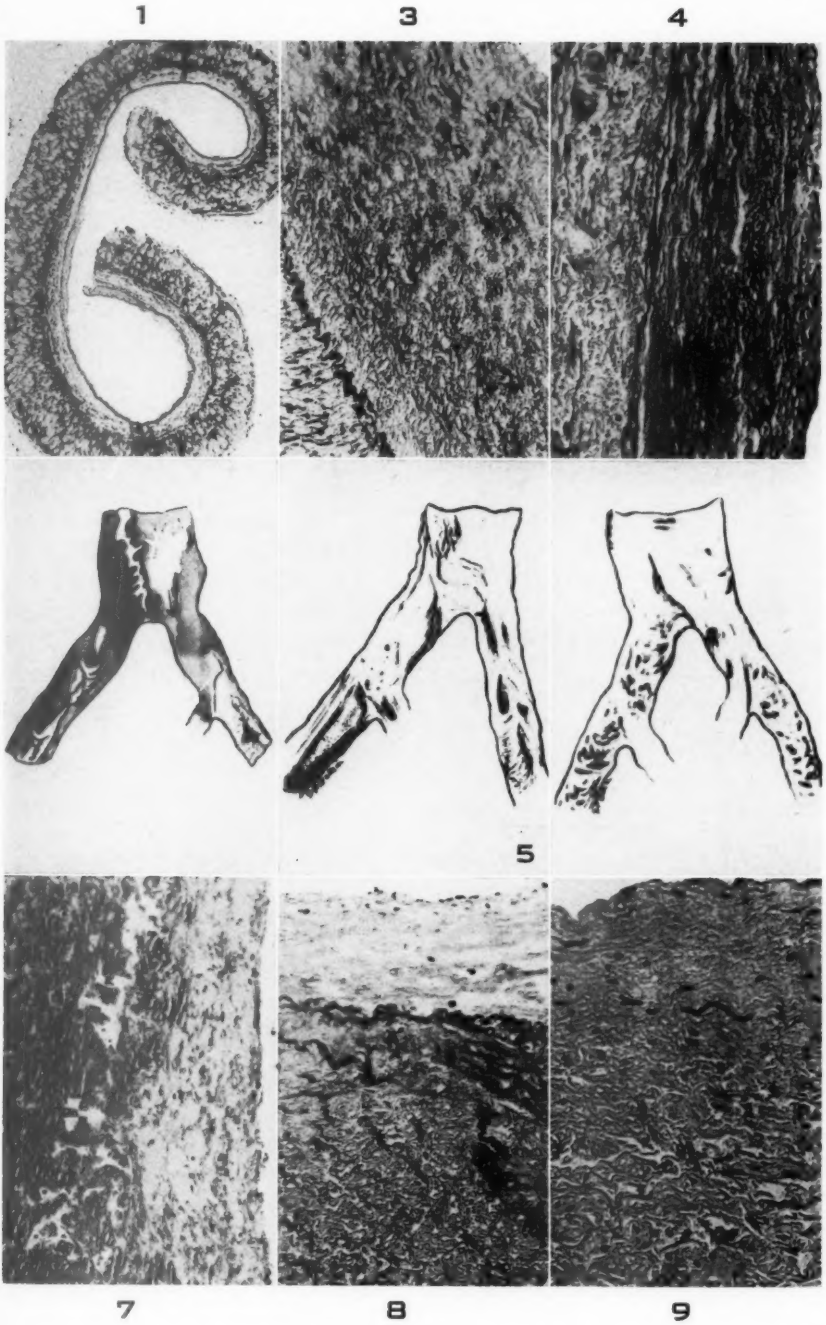
FIG. 2. Diagrammatic sketch of the lower inferior vena cava, common and external iliac veins in the second decade.

FIGS. 3 and 4. Endophlebohypertrophy in the external and common iliac veins. Weigert's elastic tissue-van Gieson stain. *Fig. 3:* Transverse section. × 100. *Fig. 4:* Longitudinal section showing predominance of elastic component. × 140.

FIGS. 5 and 6. Diagrammatic sketch of plaques in external and common iliac veins in the aged. *Fig. 5:* Without increased pressure. *Fig. 6:* With increased pressure.

FIGS. 7 to 9. Endophlebosclerosis in the external and common iliac veins. *Fig. 7:* Disruption of elastic fibrils. Weigert's elastic tissue-van Gieson stain. × 140. *Fig. 8:* Disruption of internal elastic lamellae. Weigert's elastic tissue-van Gieson stain. × 140. *Fig. 9:* Loss of muscle cells in intima. Hematoxylin and eosin stain. × 200.





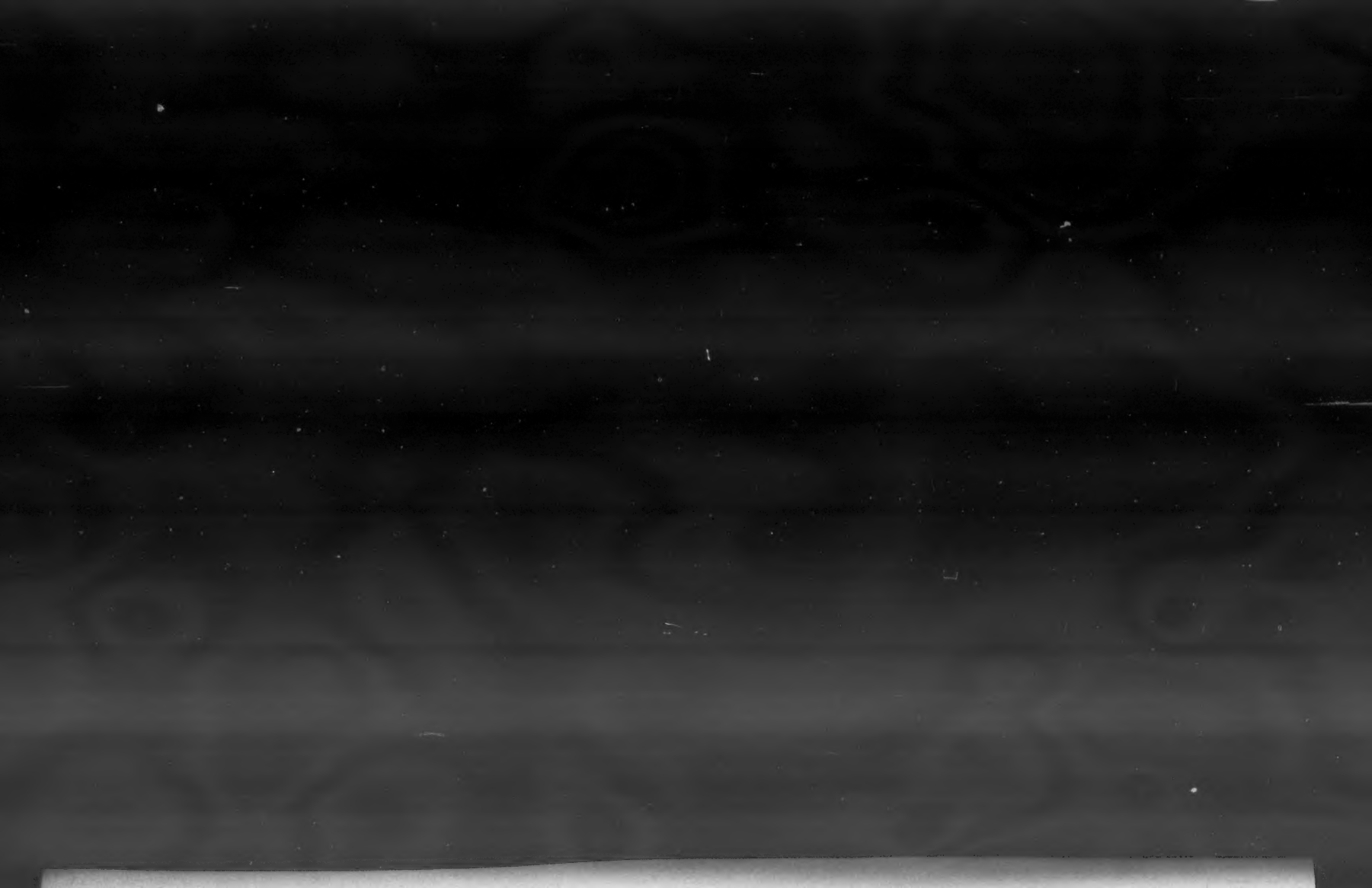
Lev and Saphir Endophlebohypertrophy and Phlebosclerosis

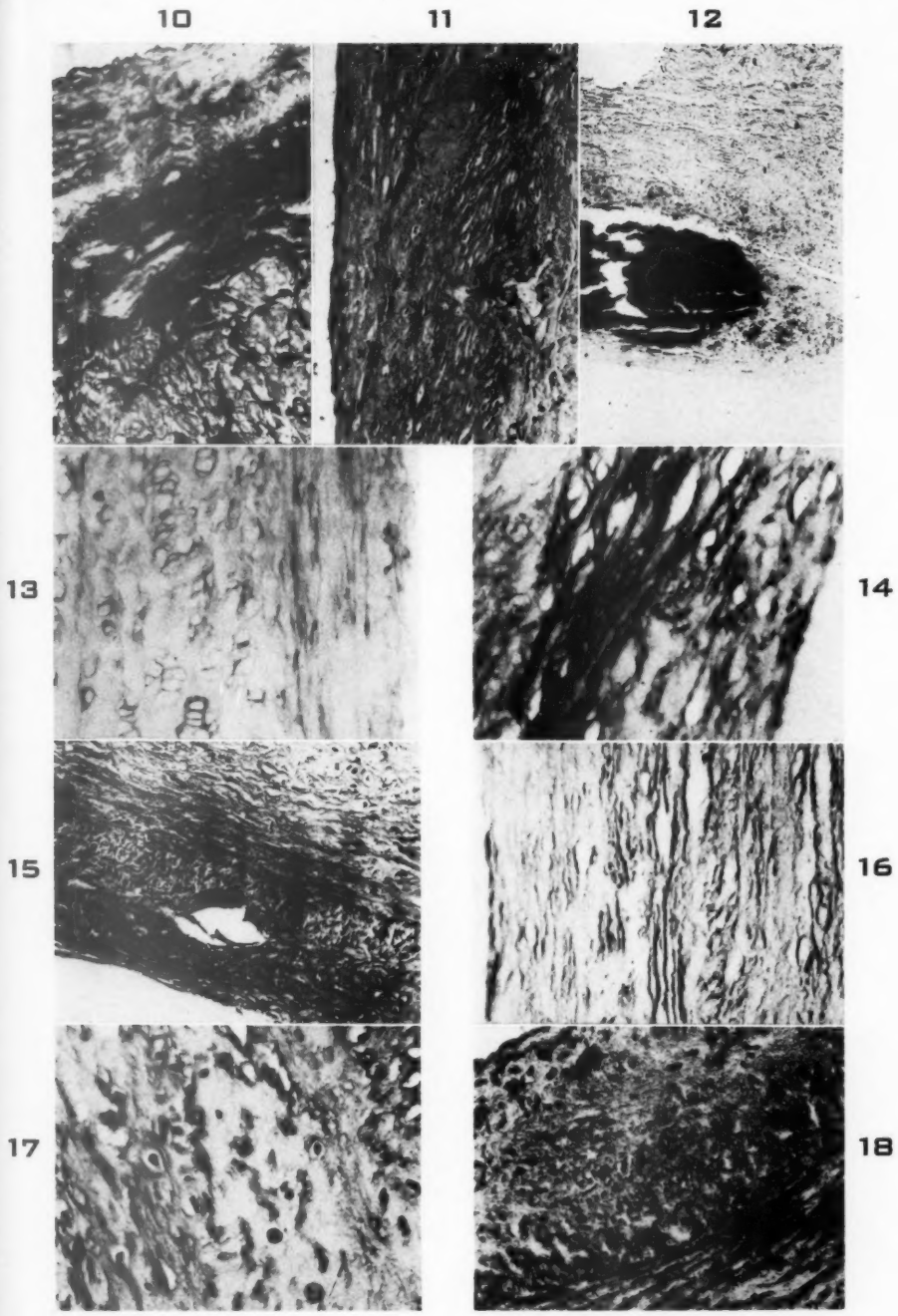
PLATE 67

FIGS. 10 to 12. Endophlebosclerosis in the external and common iliac veins. *Fig. 10:* Increase in connective tissue. Weigert's elastic tissue-van Gieson stain. × 140. *Fig. 11:* Appearance of vasa in the intima. Hematoxylin and eosin stain. × 100. *Fig. 12:* Zone of calcification in the intima. Hematoxylin and eosin stain. × 100.

FIGS. 13 to 16. Endophlebosclerosis of the iliac veins. Changes in basement membranes and ground substance. *Fig. 13:* Focal decreased staining of the glycoprotein component of the basement membranes and ground substance. McManus-Lillie-Hotchkiss stain. × 280. *Fig. 14:* Increased staining of glycoprotein component of the basement membranes. McManus-Lillie-Hotchkiss stain. × 280. *Fig. 15:* Lacunar formation in the ground substance. Hematoxylin and eosin stain. × 140. *Fig. 16:* Increased and decreased staining of the reticular component of the basement membranes. Hortega's reticulum stain. × 280.

FIGS. 17 and 18. Endophlebosclerosis of the iliac veins. *Fig. 17:* Degenerated nuclei and inflammatory cells. Hematoxylin and eosin stain. × 400. *Fig. 18:* Deposition of fat in muscle cells. Sudan IV stain. × 140.





Lev and Saphir

Endophlebohypertrophy and Phlebosclerosis

THE PATHOGENESIS OF ATHEROMA *

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Atheroma, or atherosclerosis, is the term applied to a highly characteristic lesion of arteries. The lesion is focal and is confined to the intima. It consists of a fibro-hyaline thickening with, commonly, on the deep aspect, accumulation of lipids which may be both intracellular and extracellular. While the aorta and other large arteries may be dilated, usually the lumen of affected smaller vessels is encroached upon. The classical appearances are illustrated in a frozen section of a coronary artery (Fig. 1).

Atheroma may be complicated by further degenerative changes: The plaques may calcify, may undergo superficial necrosis (atheromatous ulceration), and, especially in vessels of the order of coronary arteries, thrombosis may occur. As a late phenomenon the adjacent media may become thinned, and, where the intimal thickening is insufficient to compensate for this weakness, aneurysm follows. Perhaps as common as this saccular aneurysm formation, is a further process of surface ulceration and deep necrosis leading to a dissecting aneurysm. The lipidic accumulation is composed mainly of cholesterol and its esters and the quantity is often sufficient to impart a characteristic yellow color.

Experimental Cholesterol Atherosclerosis

Duff[1] has recently reviewed the pathogenesis of atheroma. He is the worthy protagonist of the school whose members center their argument around the rôle of cholesterol, beginning with the well founded observations that the cholesterol content of human atherosclerotic lesions is high, that the daily feeding of cholesterol to rabbits over a period of months leads to the development of cholesterol accumulations in the intima of blood vessels, and that atheroma is severe in diabetes mellitus, a condition associated with hypercholesterolemia. Duff concluded that "the deposition and accumulation of cholesterol in the intima of arteries has come to be regarded as the central feature of the disease," although it is admitted "in regard to mechanisms or dynamics of development that we are particularly ignorant."

When rabbits are fed with cholesterol, experimental cholesterol atherosclerosis results. It is evident that the rabbit's tissues treat the large quantities of cholesterol as something foreign because the cho-

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lesterol first accumulates in the reticulo-endothelial system. When this system is saturated the cholesterol is deposited in the subendothelial system of the intima of arteries. The deposition starts in the aorta and ultimately involves even the smallest arteries. When feeding is discontinued the lesions undergo retrogression. While such lesions are produced with facility in the rabbit, cats, dogs, and monkeys are totally resistant.[2,3] The preliminary saturation of the reticulo-endothelial system, the retrogression of the lesion when cholesterol feeding is stopped, the involvement of the smallest vessels, all have no parallel in human atheroma. While the experimental lesion resembles human atheroma, one can go little further. The reticulo-endothelial system can be regarded as a large, fixed phagocyte. All tissues, however, possess a potential phagocytic system which will proliferate and differentiate on suitable stimulation. This potential system is especially identified with the mesenchymal investment of endothelium. There are thus reasonable theoretic grounds to look on the intimal accumulations of cholesterol in the rabbit merely as the calling forth of a secondary phagocytic system to deal with blood-borne foreign matter which has already over-taxed the fixed phagocytic system of the organism.

"Experimental" Cholesterol Atherosclerosis in Man

I do not consider the usual atheroma in man as the morphologic equivalent of experimental cholesterol atherosclerosis in the rabbit. Nevertheless one occasionally encounters lesions in man with that distinctive structure. This is illustrated in Figure 4 of a small artery in which the lumen is reduced to capillary dimensions. The endothelium is supported by well defined, condensed collagen. Outside this, the intima is greatly expanded by lipidic foam cells. Those appearances are identical with the characteristic lipid cushion of experimental cholesterol atherosclerosis. In addition, the vessel shows other degenerative changes: the media is completely hyalinized, and there is periarterial round cell infiltration and fibrosis. I have encountered such appearances in significant number only in occasional cases of diabetes mellitus with marked hypercholesterolemia and severe hypertension. The findings suggest that in the presence of arterial damage (secondary to hypertension) and hypercholesterolemia, local conditions obtain which can cause uptake of cholesterol from the blood into the intima in a manner which corresponds to experimental cholesterol atherosclerosis.

The Encrustation Hypothesis of Rokitansky

A rival theory to the cholesterol school was first propounded in 1852 by Rokitansky[4] who concluded that atheroma was produced by the

deposition of "an endogenous product derived from the blood, and for the most part from the fibrin of the arterial blood." This theory was eclipsed only 4 years later by Virchow[5] whose teaching that atheroma was essentially an intimal degeneration followed by superficial fibrous thickenings has remained current. Duguid[6,7] recently has reinvestigated the question and resuscitated the original theory of Rokitansky.

In his first communication, Duguid[6] dealt with atheroma of the coronary arteries. He showed with elegant illustrations prepared from frozen sections how the common intimal thickenings, producing the characteristic eccentric or often crescentic, reduced lumen, can be built up by successive laminae of different ages. These laminae, thick enough to be visible with low-power microscopy, relate to recurrent episodes of mural thrombosis and subsequent organization. Duguid also pointed out that red thrombi are prone to undergo fatty change. To counter the argument that he had chosen a site where thrombosis complicating atheroma is common, Duguid, in his second paper,[7] dealt with the organization of wisps of fibrin, fibrinous encrustations, by the intima. This study concerned the aorta and he showed how minute coagula, composed mainly of fibrin, are deposited on the endothelium and incorporated in the intima by overgrowth of endothelial cells. With inspissation and organization the encrustations merge into the glassy groundwork so common in intimal thickenings. Thus, according to Duguid, focal intimal thickenings can arise and progress by repeated organization of microscopic deposits of coagulum from the circulating blood, while, at least in the coronary arteries, more extensive thrombosis, again often recurrent, leaves distinctive laminations in an accentuated build-up of the focal intimal thickenings. The rich lipid content is attributed partly to the breakdown of incorporated red cells. It is difficult not to accept Duguid's illustrations at their face value, but it has not been determined how frequently the evidence presented by Duguid would be obtained in a large random series of lesions from various arteries. Certainly such evidence may not be visible, although, as is shown in Figure 1, the protagonist of the Rokitansky view could point out that some red cells are adherent to the endothelium. Their slow assimilation to the intima over the years could build up the thickening which we see. Duguid's hypothesis, in the manner in which it is demonstrated from actual sections, is illustrated diagrammatically in Figure 2.

Intimal Vascularization in Atheroma

The subject of intimal vascularization in atheroma was dealt with in an important monograph by Winternitz, Thomas, and LeCompte.[8]

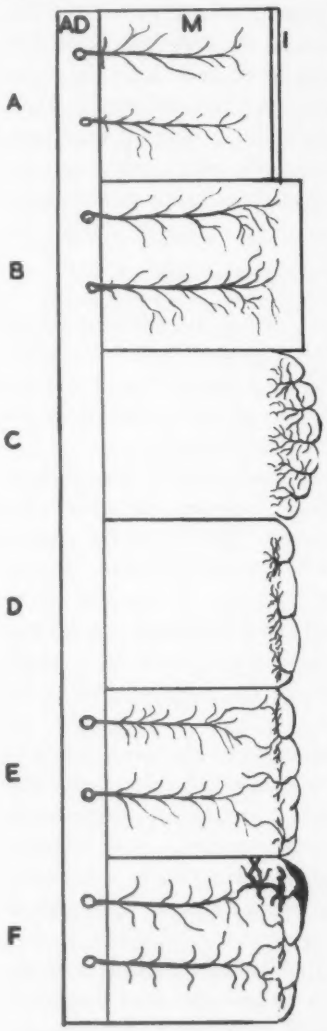
It may be that the lessons of their large series of exquisite illustrations have been neglected because they went too far in claiming for intimal vascularization a decisive rôle in the genesis of atheroma. A recent paper by Geiringer[9] contained important emendations of their work and went far toward bridging their work with Duguid's contributions.[6,7] This is illustrated in a diagrammatic representation of part of the wall of an artery (Text-fig. 1).

How much of the concepts presented in Text-figure 1 is theory? The phenomenon of vascularization of intimal thickenings has been known since the time of Rokitansky.[4] Winternitz *et al.*[8] provided abundant illustrations showing all of the types of vascularization illustrated in the diagram and asserted that intimal vascularization is as common as atheroma itself. Geiringer[9] claimed that he could find evidence of intimal vascularization in 90 per cent of intimal thickenings with the examination of only a single routine section in each case. We can follow also Geiringer's statement that: "Secondarily evolved blood supplies occurring in pathological new formations are notoriously unstable and subject to interference. They are built up haphazard in response to local need and lack the safeguards which characterize the more hereditarily determined plexuses of normal organs. Often they run in abnormal tissues which do not provide them with sufficient support. They lack good collateral connections and shunts and are built on a marginal basis. The component vessels are often of the giant capillary type, *i.e.*, with walls inadequate for the volume of blood which they carry. An intima depending on such a precarious blood supply is prone to secondary disturbances which, because of the proximity of the arterial lumen, may not be confined to this tissue but have serious local and distant repercussions."

We must also allow that Winternitz *et al.*[8] have demonstrated thrombosis and hemorrhage as vascular accidents in the adventitious blood supply of atheroma. Geiringer[9] is probably correct in interpreting the hemorrhage as secondary to micro-infarction, and in explaining the causal vascular obstruction, when not obviously thrombotic, as being secondary to either sudden lowering of pressure or vasospasm, and perhaps mediated by the adventitious vasculature having been inadequate from the outset.

It must be obvious, with three possible methods of adventitious vascularization, with combinations and imperfections of these, with different vascular accidents which might lead to pale or red, firm or soft, micro-infarction, and with recurrent episodes of complications and resulting reparations, that close analysis of the lesions of the intimal vasculature in atheroma presents great difficulties. Geiringer[9]

stated that: "This type of intimal vascularisation [transmedial vascularisation] is found most often where the intima has thickened by slow degrees and in a more or less concentric fashion. It is, so to speak, the 'normal' method by which the intima acquires an adventitious



Text-figure 1. Patterns of intimal vascularization. A illustrates the all important vasa vasorum which arise in the adventitia (AD) and vascularize the media (M) to a critical depth, ending approximately at the junction of the middle and inner thirds. The inner third of the media and the intima (I) are non-vascular and receive nourishment by diffusion from the blood in the lumen.

B illustrates the reaction, constant under normal conditions, to any slowly progressive intimal thickening. This thickening leaves a zone outside the effective range of diffusion from the lumen; the resulting tissue anoxia provides the stimulus to ingrowth of the vasa vasorum to the depth shown. *This is transmedial vascularization.*

C shows a recent mural thrombus on the intima. It is, as always, richly vascularized by the ingrowth of channels which communicate with the lumen.

D represents advanced organization and shrinkage of the mural thrombus of C. An adventitious blood supply persists to supply the deep part of the thickened intima which is beyond the nutritional field of the circulating blood. This adventitious vasculature is characteristic, producing an "inverted tree" appearance, since short vascular stalks run from the endothelial surface to arborize on the deep aspect. *This is vascularization from the lumen.*

E represents a combination of luminal and transmedial vascularization associated with thrombogenic intimal thickening and entailing anastomosis between the two systems. *This is the combined form of vascularization.*

F. At X there has been a vascular accident in the combined form of vascularization, resulting in local micro-infarction. Diffusion of thromboplastins from this area has resulted in further deposit of thrombus on the thickened intima.

blood supply, since it is derived from the normal peri-arterial plexus. It is seen most frequently in the fibrous hyperplasia without marked atheroma so often encountered in the coronary arteries of aged men." In this we can follow him. He also stated that "Plaques of atheroma also may show this variety, but more often display the second type of intimal vascularisation [vascularisation from the lumen]." Since vascularization from the lumen is the hallmark of thrombogenic origin, this would indicate that a majority of atheromas, or at least the major portion of the thickening in most atheromas, is of thrombogenic origin. This point is so important and the analysis is so difficult that more verification must be awaited. Nevertheless, the work has magnified the importance of thrombogenesis as an important factor in the pathogenesis of atheroma.

Experimental Mural Encrustations

Clearly the time has come to evaluate Duguid's contentions[6,7] by the experimental method. So far only scant information is available. Harrison[10] introduced fibrinous emboli into the pulmonary circulation of rabbits. The end result of their impaction and organization in the branches of the pulmonary artery is an intimal elastosis.

Three years ago I approached the problem, and, since I have not been able to return to the work, the observations made are presented with the speculations which the work engendered. The intention was to seek some mechanism which would produce showers of small thrombi in the pulmonary arterial system. While it is easy to produce intravascular coagulation by slowing the stream and damaging the intima, it is notoriously difficult to produce coagulation by methods which alter the circulating blood without introducing large quantities of foreign material.

The venom of the Russell viper is reputed to be the most powerful coagulant known. In dilutions which will markedly accelerate the coagulation of blood *in vitro* the available proprietary preparation of the venom (stypven*) gave disappointing results. It is believed that the action of the venom is enhanced by the action of a lipid cofactor[11] and, accordingly, a rabbit brain extract rich in lipid (bacto thromboplastin†) was combined with stypven. Eventually it was found that the following method gave reliable results with rabbits. Stypven was used in dilutions of one in a million and thromboplastin one in fifty in sterile distilled water. One ml. of each solution was employed and they were either injected simultaneously into separate ear

* Burroughs Wellcome & Co., New York, N.Y.
† Difco Laboratories, Detroit, Mich.

veins or in immediate sequence without removing the needle. It was presumed that the solutions mix in the right heart before entering the pulmonary circulation. One would expect the main effect of the coagulant to be in the pulmonary arterial system. Indeed, I found changes only in this system, apart from secondary changes associated with right heart failure in a few rabbits which had widespread blockage of the pulmonary arteries.

Single Injections

Rabbits, 12 in all, were given a single injection and killed at intervals from hours to 6 weeks. The treatment sometimes produced typical red thrombi which lodged in branches of the pulmonary artery with results closely similar to those described by Harrison[10] for fibrinous emboli. In these we are not, for the present, especially interested; they will be mentioned later.

There was also evidence that the impaction of emboli induced vasospasm in the pulmonary arterial tree, as has been demonstrated also by Harrison.[12] This was seen in the lungs of rabbits killed shortly after an injection. It was found, on cutting frozen sections, that some branches of the pulmonary artery were irregularly swollen and contorted. The walls of affected segments showed edema and slight round cell infiltration with narrowing of the lumen. There was also edema and round cell infiltration in the surrounding interstitium. In the illustration provided, the rabbit had received two previous injections (Fig. 3).

In addition, the injection treatment produced a thin coating of thrombus over varying areas, sometimes large, of the endothelial surface of main branches of the pulmonary artery. This consisted of little more than a single layer of red cells with eosinophilic coagulum which adhered to the endothelium. The intimal reaction associated with the coagulum was much more conspicuous. The endothelial cells became swollen, proliferated, and within 48 hours had grown over the coagulum and disappeared. After 4 days there was left an edematous intima in which fibroblasts were prominent and the endothelial cells were still plump and occasionally in two layers. Associated with this diffuse reaction there were sometimes small focal accumulations of red coagulum. Ultimately the intima returned to normal or there was a slight increase in elastic tissue.

Repeated Injections

Groups of rabbits were injected once weekly for 3 to 6 weeks. Some were killed at varying intervals up to 3 months. Others were rested for 6 weeks and then given a second similar course and killed at inter-

vals up to 7 months from the start of the experiment. In all, 36 rabbits were used. Six were rejected because of gross thrombosis of main branches of the pulmonary artery, or of widespread thrombosis of smaller branches. The remainder showed the results of repeated organization of red coagulum by the intima.

Diffuse Lesions. The coagulum in those killed within 2 days of the last injection was generally more conspicuous than in rabbits with single injections but of the same order, namely, a diffuse thin lamina of red cells and eosinophilic coagulum. Fibrin threads and platelet masses were identified in the coagulum. While with repeated injections one could not identify the ages of all lesions, the series was sufficient to build up a picture of the process. The initial response to the coagulum was the same as in the first series, but with repeated reactions the intima became thickened by fibrocellular connective tissue. The cells were either fibroblasts or were not sufficiently differentiated to declare other than that the proliferation represented fibroplastic response of sub-endothelial mesenchyme, with some histiocytic differentiation. The endothelial cells were plump, loosely arranged, and sometimes formed more than one layer. They grew over the red coagulum and the details of the coagulum were soon lost leaving a thickened fibroplastic intima in which the coagulum had merged with a glassy ground substance in the surface layers.

Focal Lesions. Focal lesions were more numerous and larger following repeated injections. Some could be identified with the naked eye as bright yellow spots on the intima of main branches of the pulmonary artery. The yellow spots were produced by clumps of red coagulum which had been incorporated in the intima by overgrowth of endothelial cells. The coagulum, largely composed of conglutinated red cells, was degenerating into a mass of fatty material (Figs. 5 and 6). It is important to realize that the hemoglobin of red cells in arterial thrombi is rapidly washed out into the circulation. In addition to the yellow spots there were many smaller fibroplastic intimal thickenings; some contained fat but generally only as minute flecks. Fat was not demonstrated in the majority (Figs. 7 and 8). On the surface of the plaques endothelial cells were prominent and heaped-up (Figs. 7, 8, and 9). The endothelial proliferation could be seen in earlier lesions to develop as a reaction to adherent red coagulum.

Organization of Lesions. The diffuse and focal lesions completed their organization within 6 to 8 weeks. The endothelium returned to normal. The diffuse and small focal intimal thickenings diminished. Though collagen was laid down, elastic tissue appeared early and soon predominated, finally producing intimal elastosis (Fig. 10). Sometimes

this split to enclose a collagenous lamina (Fig. 11). Occasionally, small amounts of fat were present in this layer.

The prominent fatty lesions of the order shown in Figure 5 ultimately resolved to produce fibro-fatty intimal thickenings. While a few differed from atheroma in that they contained increased elastic tissue, in most of the larger lesions increase of elastic tissue was not a feature. The latter lesions were indistinguishable from human atheroma in that they presented as focal fibrous thickenings confined to the intima, with a deposit of lipid on the juxtamedial aspect (Figs. 13 and 14).

When an animal was rested after a series of injections and then killed shortly after a new injection, combinations of acute upon chronic lesions were identified. By studying those lesions it was clear that pre-existing intimal lesions were more prone to recurrent deposition of thrombus than previously unaffected intima.

Gross Thrombosis. I wish to emphasize that the preceding description was based on mural thrombosis and organization in the main branches of the pulmonary artery up to branches of a segment. In those processes there was never any question of complete or nearly complete blockage by thrombus. Reference has been made to rabbits which were rejected because the injection had produced gross thrombosis in a main vessel or in many smaller branches. As might be expected with repeated thrombosis of different degrees and ages, occurring at different levels of the arterial tree, the appearances of organization were bizarre and complex. The findings, however, have some bearing on the subject. Where vessels had been completely obstructed, particularly in smaller branches, the end result of organization and recanalization was an intimal elastosis similar to that described by Harrison[10] for the organization of fibrinous emboli. But sometimes in larger branches a mass of red thrombus had undergone fatty softening; with recanalization and the laying down of connective tissue from the intima, the fat appeared juxtamedial leaving appearances similar to atheroma. In addition, bizarre filiform and polypoid red thrombi had become attached by a narrow base to the intima of some large vessels. These were rapidly organized. While ultimately mainly fibro-elastic, two contained osteoid tissue. This is mentioned later under Lambl's excrescences.

Conclusions as to Experiments

The experiments which I have described indicate that the organization of recurrent minimal layers of red thrombus in the pulmonary arterial system leads eventually to intimal elastosis. Likewise, with

more marked and focal deposition of red thrombus the end result is usually a fibro-elastic plaque. The intimal reaction for red thrombus is thus not different from the reaction described by Harrison[10] for fibrinous emboli. Similar results were found when red thrombus had filled an artery. Since intimal elastosis is not a feature of atheroma, the results thus far discussed do not support Duguid's[6,7] contentions on the pathogenesis of atheroma.

Mural deposits of red coagulum in large vessels were organized to form fibro-fatty intimal thickenings, and these were sufficiently free from elastosis to be indistinguishable from human atheroma. Within the limits of the experimental series, the results pointed to the red thrombus-endothelial surface ratio as having a determining influence, since it was focal lesions of the order of Figure 5 which organized to produce atheromatous plaques. In lesions in small vessels and in diffuse and plaque-like lesions in large vessels, the reacting intima appeared to be able to deal with the red coagulum without leaving a fatty residue. In lesions of the order of that of Figure 5, where the field of reacting endothelium was small relative to the size of the coagulum, the mass of red coagulum was visibly incorporated as such in the intima by endothelial overgrowth. Another possible factor which could not be properly assessed is stress. Thus, while most of the rabbits did not show signs at necropsy of embarrassment of the pulmonary circulation, a temporary phase of pulmonary hypertension in any animal was not excluded and no doubt it occurred in most from occlusion of small vessels and vasospasm.

The results with minimal diffuse coagula or focal coagula did not differ radically from complete obstruction with red thrombus. Here again the end result of organization was intimal elastosis although occasionally in large vessels fatty changes *en masse* in the red thrombus appeared to produce changes indistinguishable from atheroma. None of these, however, were followed further than intermediate stages of organization.

The field of speculation which these experiments open up appears to be more important than the actual results. Blood is fluid within the vascular system during life by virtue of a dynamic equilibrium between anti-coagulant and coagulant mechanisms. When coagulants are injected into the vascular system, anticoagulant mechanisms are presumably stimulated since results are often disappointing as compared with the reaction *in vitro*. It is generally assumed that these mechanisms are inherent in the circulating blood, although the rôle of some tissue anticoagulant factors, *e.g.*, heparin, is obscure. The vascular endothelium is generally considered to play a passive rôle with its

physical property of relative non-wettability. I have doubts about the confinement of the endothelium to a passive rôle.

Active proliferation of the endothelium was the invariable reaction to a coating of thrombus. The more marked the reaction the more difficult it was to detect the thrombus. It could be identified in its diffuse form by studying the vascular system carefully cleared of circulating blood by perfusion with saline solution before fixation; but within 36 hours its place is marked by proliferating endothelium. While repeated injections resulted in a fibroplastic thickening of the endothelium indicating an organizing process, the trend of the experiments suggested to me that the endothelium, up to a point, is actively thrombolytic and that the focal fatty lesions represent areas where this action is overwhelmed by the mass of the coagulum. The question of the endothelium producing a fibrinolytic enzyme has been raised on other evidence.[18]

It is a valid basis for criticism of the experimental procedures to suggest that the injection may have produced non-specific damage to the endothelium and that thrombosis is secondary. No doubt the investigation requires more elaborate treatment, but I have drawn no conclusions which would be vitiated by such a criticism. It is no argument to say that the results are not relevant to the problem of atheroma since the lesions were produced in the lesser circulation. One is testing only the reactions of endothelium in general. In attempting to produce the lesions in the systemic circulation I have used intracardiac injections, but these always set up a fibrinous pericarditis.

No similar lesions were found in the control animals. In aged rabbits, outside the scope of the experiments, it was found that small pulmonary emboli from the leg veins were not uncommon. These gave rise eventually to focal intimal thickenings mainly composed of elastic tissue.

Localizing Factors. One of the major obstacles to acceptance of Rokitansky's[4] hypothesis rests in the fact that atheroma does not occur in veins, while one would reasonably expect mural encrustations to be more common in veins than arteries, since gross thrombosis is more common. Whatever experimental approach is used, the investigator will be faced with this and other problems of localization. Not only is atheroma not found in veins, but the lesions occur in the lesser circulation only when there is longstanding hypertension, as in some cases of mitral stenosis, and, though widespread in the systemic circulation, the smallest vessels escape. Stress has long been considered an obvious factor. The lesions tend to be severe in hypertension, occur in the lesser circulation under hypertension, and in their ultimate dis-

tribution local stress appears to be a factor. Witness the ring-like lesions around the aortic orifices of the vertebral arteries. It appears obvious that little advance can be made without more precise knowledge of the general biology of blood vessels, in particular their reactions to stress and the relationship of the endothelium to the normal fluidity of the blood. It is noteworthy that the thrombogenic lesions which I produced were often conspicuous around the orifices of branches (Fig. 12).

Pyridoxine Deficiency. Rinehart and Greenberg[14] reported focal intimal thickenings in primates suffering from experimental pyridoxine deficiency. Their illustrations resemble many of the intimal thickenings which I have observed in my experiments and it will be interesting to see an evaluation of their results in relation to coagulant mechanisms.

Other Fibrinous Encrustations. Duguid's revival[6,7] of Rokitansky's[4] original theory has not been without results in other fields. Lambl's excrescences are minute filiform projections found on the mitral valves. These have recently been shown to result from the organization of fibrinous thrombi.[15] Similar findings in arteries are reported in my experimental series. Rokitansky held also that the progressive thickenings of the valves in chronic rheumatic disease resulted from the progressive deposition and organization of fibrinous deposits from the blood on the damaged valve. This view has recently been substantiated.[16]

Buerger's Disease. When young rabbits are given repeated daily intravenous injections of the coagulant mixture which I have described, gross thrombosis ensues in the pulmonary arteries. This is followed by a brisk endothelial and intimal proliferation such as I have described previously. In addition, with the severe degree of thrombosis, there is generalized edema and leukocytic infiltration of the media and marked perivascular inflammatory response. The late results of organization show the lumen of the vessel partly recanalized by capillary channels. The tissue replacing the intima is mainly elastic. The media is fibrosed and there is slight perivascular fibrosis. Most limbs amputated with a diagnosis of Buerger's disease show chronic vascular disease limited to atherosclerosis, often obviously complicated by recurrent thrombosis, and sometimes with late venous thrombosis interpreted as secondary to the ischemia of the arterial disease. The experimental results which I describe raise the issue as to whether the minority, to which a label of Buerger's disease is applied, are not the result of a similar process in which the reaction of the vessel wall

to the original thrombosis has been of an enhanced degree, and in which vasospasm plays an important part.

General Considerations

Cholesterol Atherosclerosis. It is considered that the mechanism of experimental cholesterol atherosclerosis does not play a part in the primary genesis of the generality of atheroma in man. Occasionally, under conditions of vascular damage and hypercholesterolemia, a mechanism may come into play which locally is similar to the experimental mechanism. Within the limits of this argument we may speak of cholesterol atherosclerosis. At present it must be regarded as exceptional and, since the nuances of vascular damage and cholesterol derangement which permit it are not known, we can only hazard a guess that the mechanism may sometimes play a secondary part in atheroma in general.

Thrombogenic Atheroma. We must accept, from Duguid's illustrations,[6,7] that atheroma can be cast in its own image by the organization of recurrent, relatively gross, mural thrombi. No one, however, seriously believes that atheroma is commonly developed by episodes of relatively gross thrombosis. In human pathology the rôle of thrombogenesis must stand or fall with the question of microscopic coagula, fibrinous encrustations, and with further enquiry into the application of the work on the collateral circulation. Herein we have an attractive hypothesis: one can assume that the blood in the circulation is not perfectly fluid and that the endothelium is not perfectly non-wettable. With the increasing imperfections of age an increasing deposit from the circulating blood upon the vascular endothelium is to be expected and hence an increasing build-up of intimal thickenings by slow assimilation and organization of the deposit. We have also the tenet of Winternitz that it is not so much that a man is as old as his arteries, but that he is as young as the adventitious vasculature in the walls of his arteries. That is to say, fibrinous encrustations vary ultimately in their disposal with the efficiency of mural vascularization. And prior even to this mechanism we have to consider whether vascular endothelium can, up to a point, actively participate in the dissolution of small thrombi. While much remains to be done, a purely thrombogenic basis of atheroma must receive serious consideration. At present one can always halt this train of thought by asking why atheroma is not encountered in veins. It may well be that the absence of the necessary stress is a determining factor in excluding the lesions from veins since thrombogenic atheroma is taken to be an expression of the failure of

normal physiologic mechanisms. All of which points to the necessity of more fundamental knowledge of the biology of blood vessels, in particular their reactions to stress.

Experimental Atheroma. In the experiments which have been described, the reactions produced were by no means simple, involving vasospasm, probable phases of pulmonary hypertension, and complete blockage of small vessels with the opening of collateral channels. No doubt they require more extensive treatment. Nevertheless, lesions indistinguishable from atheroma were produced. The critic will, perhaps, be concerned with the finding of elastosis in the small vessels and, at times, in the larger vessels in association with recurrent diffuse minimal thrombosis. In fairness to Duguid's[6,7] work it should, however, be pointed out that we are dealing in these experiments with the acute reactions of the healthy pulmonary vasculature of rabbits to mural deposit, while Duguid dealt with what is altogether a slower process in the systemic circulation, and which may well be morbid from the outset. It is suffice to state that in an experimental procedure in which red thrombus was primarily involved, and in which stress might have been a contributing factor, lesions indistinguishable from atheroma were produced. While diffuse intimal elastosis also resulted, this was associated with a relatively greater field of endothelium reacting to the red thrombus. Further experimentation may well yield other important mediating factors: it may well be that intimal thickening, occurring during a phase of pulmonary hypertension, determined the elastic nature of the thickening. For the present the advocates of cholesterol go through verbal and mental gymnastics trying to show that their work has a bearing on atheroma, while the protagonists of thrombogenesis go through a reverse process minimizing the evidence which is going so far to take atheroma out of the limbo of degenerations.

The experimental work described was carried out when I was pathologist to the Regina General Hospital, Saskatchewan. Acknowledgment is made to Miss Margaret Klassen, R.T., who carried out the technical work.

The photomicrographs of experimental atheroma are by Mr. Brian Leddy, Prairie Regional Laboratory of the National Research Council, Saskatoon, Saskatchewan. The others are by Mr. W. Merrieman, Halifax, Nova Scotia.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

All frozen sections were stained with hemalum and Sudan IV.

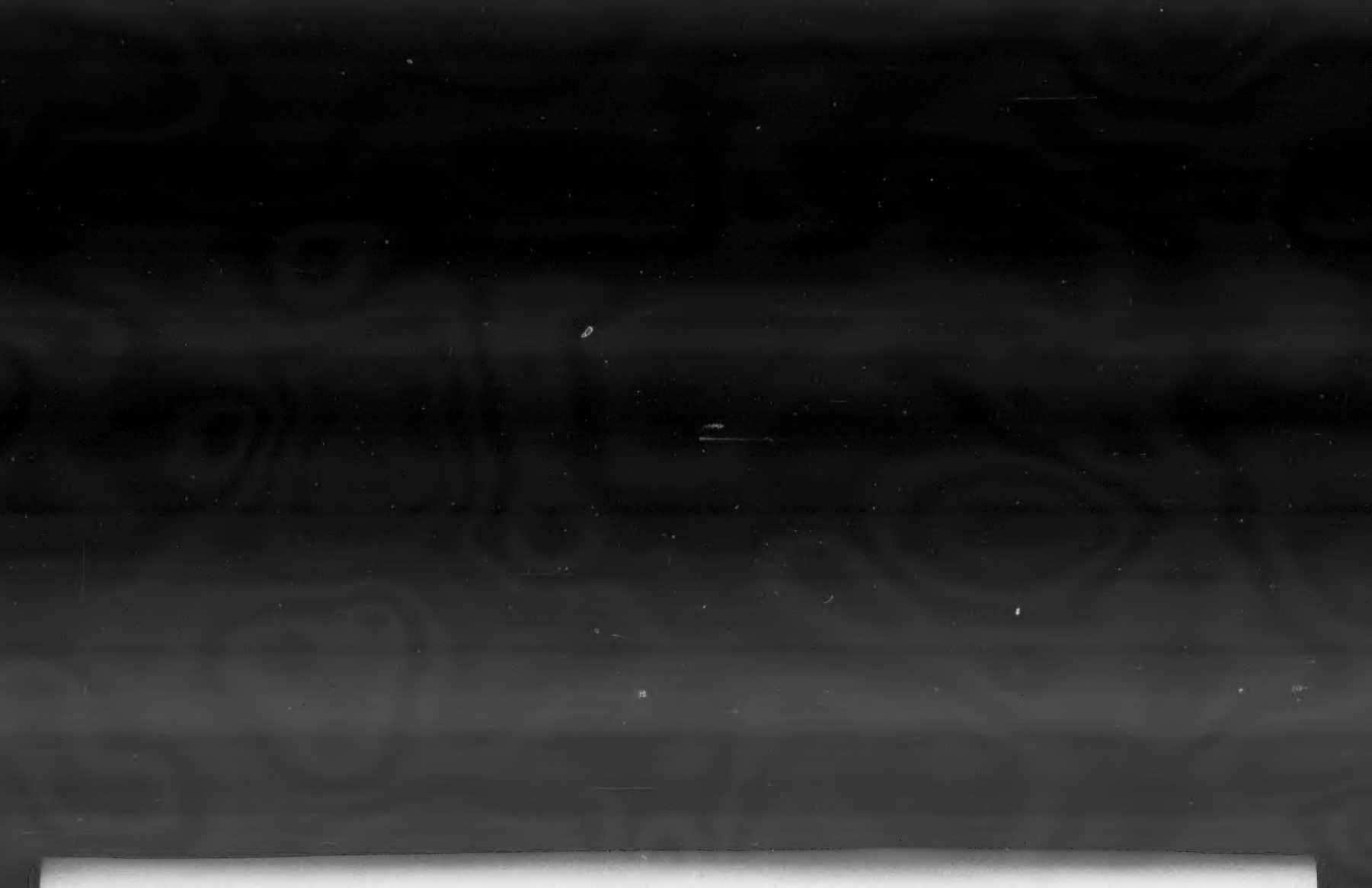
PLATE 68

FIG. 1. Atheroma of coronary artery. The pale, circular band is the media. The lumen is reduced and eccentric. The dark material in the thickened intima is lipid and is seen to be maximal in the juxtamedial aspect. Frozen section. × 10.

FIG. 2. Thrombogenic atheroma, after Duguid. A. A fibrinous encrustation has been carried under the intima. B, C, and D. Distinctive laminae relating to episodes of thrombosis and organization. B is composed of a relatively recent mass of fibrin with organization at the periphery. C is a lamina of hyaline connective tissue. D is a lamina of connective tissue which shows calcification (black) and a mass of sudanophilic sludge containing cholesterol crystals. L is the lumen.

FIG. 3. Spasm in a pulmonary artery. Frozen section. × 95.

FIG. 4. Cholesterol atherosclerosis. Paraffin section. × 345.



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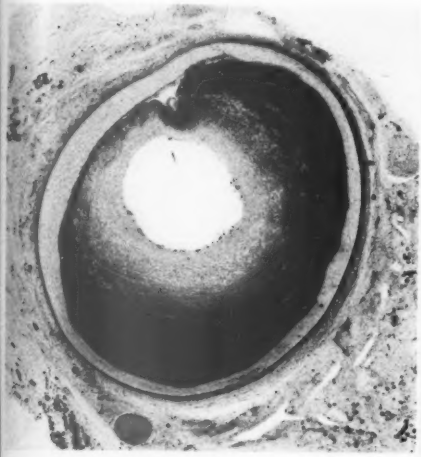

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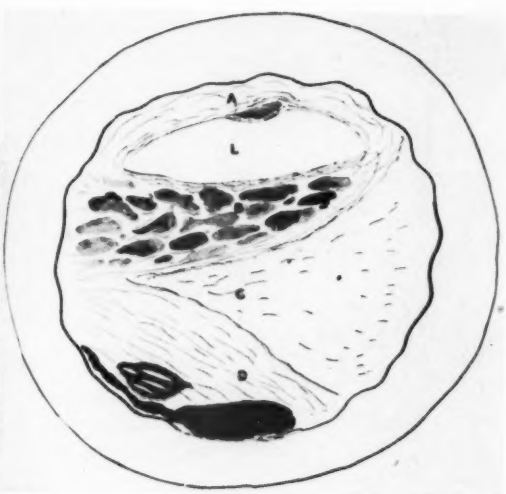

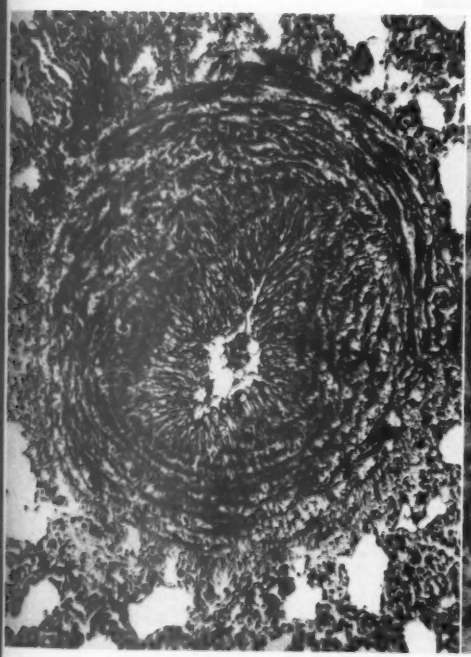

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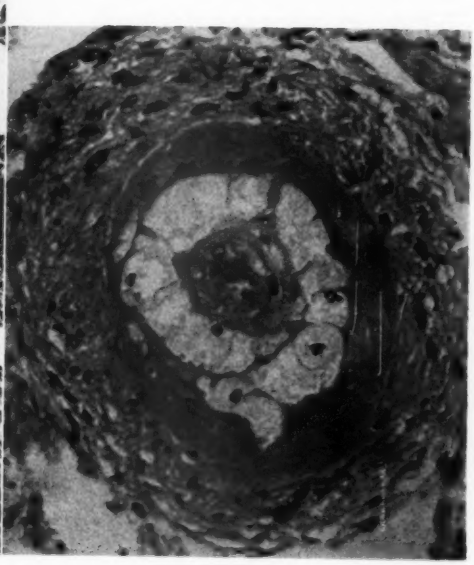

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Pathogenesis of Atheroma

PLATE 69

FIG. 5. Irregular fibroplastic intimal thickenings with two raised nodules containing fat (black in section). Frozen section. × 30.

FIG. 6. An enlargement of an intimal thickening of Figure 5 showing the sudanophilic sludge. Frozen section. × 175.

FIG. 7. Diffuse and plaque-like fibroplastic intimal thickenings. The dark surface areas are masses of endothelial cells; see Figure 8 for a higher magnification. Frozen section. × 30.

FIG. 8. Intimal plaque; of note is the thick covering of endothelial cells. Frozen section. × 160.

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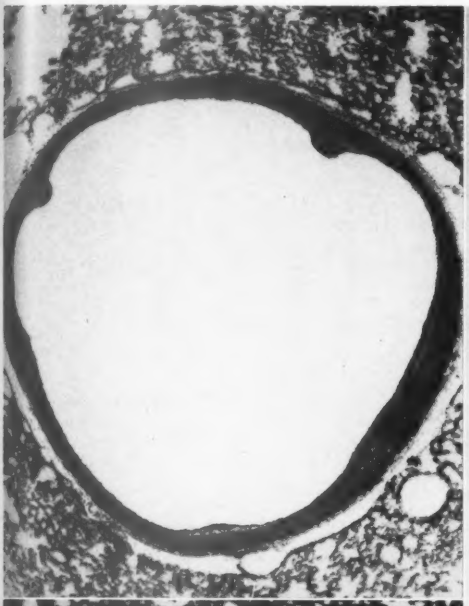

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Pathogenesis of Atheroma

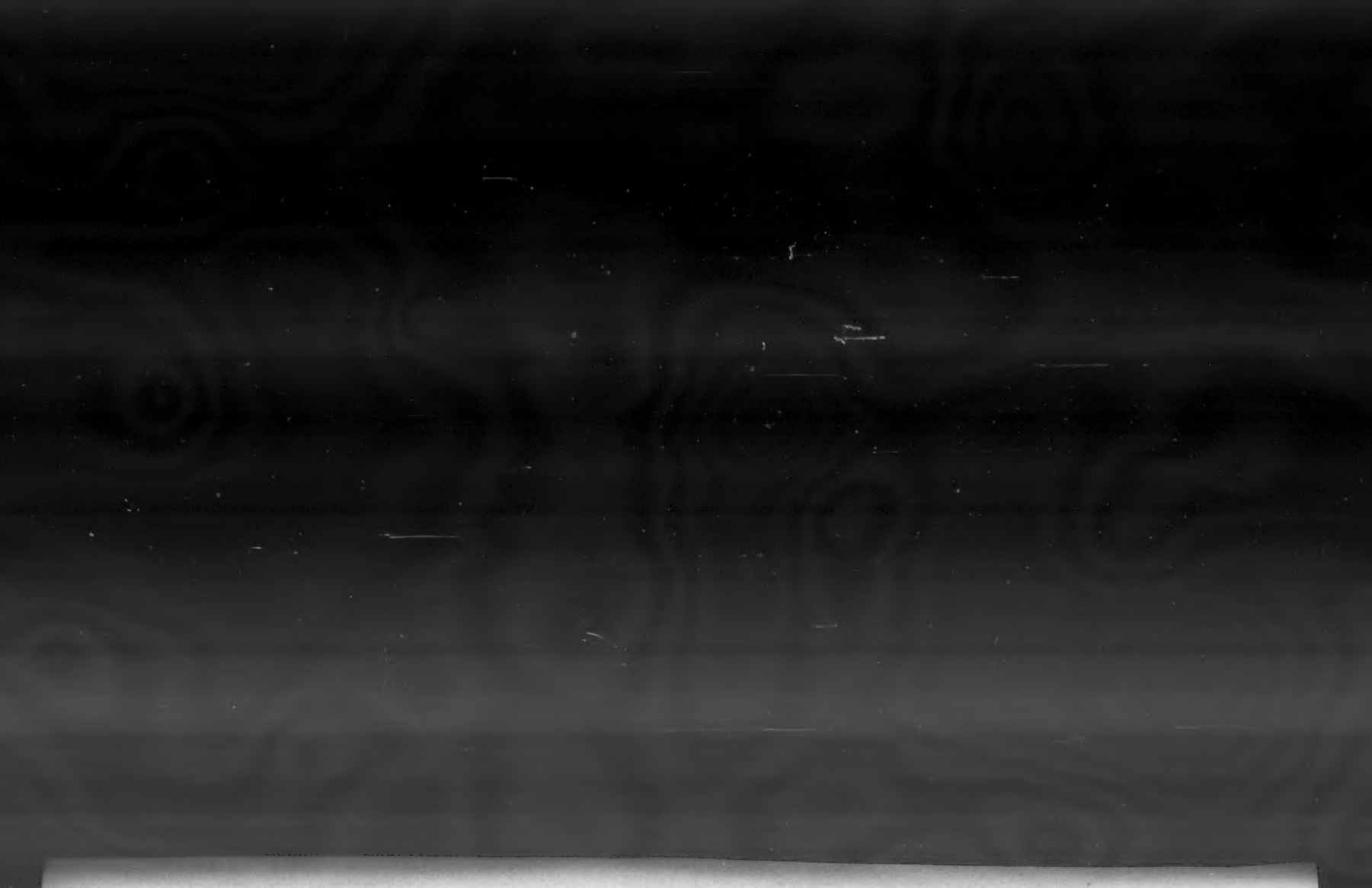
PLATE 70

FIG. 9. The intimal thickening is demarcated on the surface by a dark line representing endothelial proliferation, while the dark line on the deep aspect represents the internal elastic lamina. Frozen section. × 30.

FIG. 10. Diffuse intimal elastosis. The elastica appears black. Paraffin section. × 80.

FIG. 11. Diffuse and plaque-like intimal elastosis. The plaque is split to enclose a collagenous (pale) lamina. Paraffin section. × 130.

FIG. 12. Thrombogenic cellular lesion around orifice of branch vessel. Frozen section. × 25.



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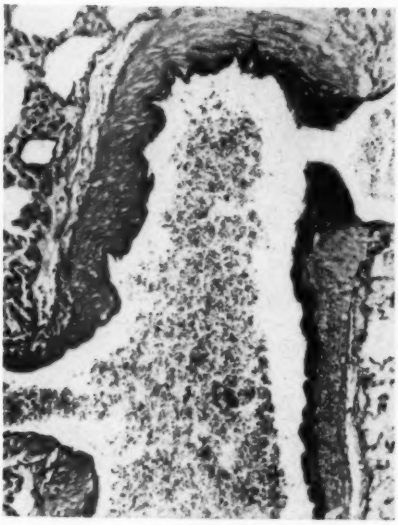

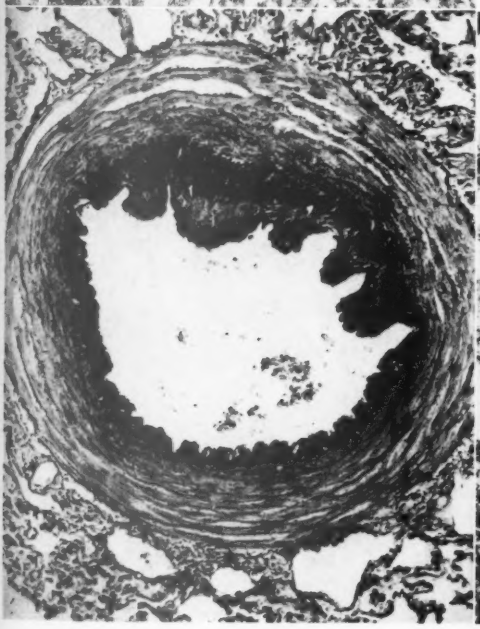

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Pathogenesis of Atheroma

PLATE 71

FIG. 13. Typical atheromatous plaque. On the deep aspect there are numerous foam cells which are vacuolated due to the solution of lipids in preparation of the section. Some adherent coagulum appears to be lifted off the intima due to histologic shrinkage. Vessel of same order as that of Figure 5. Paraffin section. × 255.

FIG. 14. Atheromatous plaque, cellular and fibrohyaline; scattered foam cells are present. Paraffin section. × 150.

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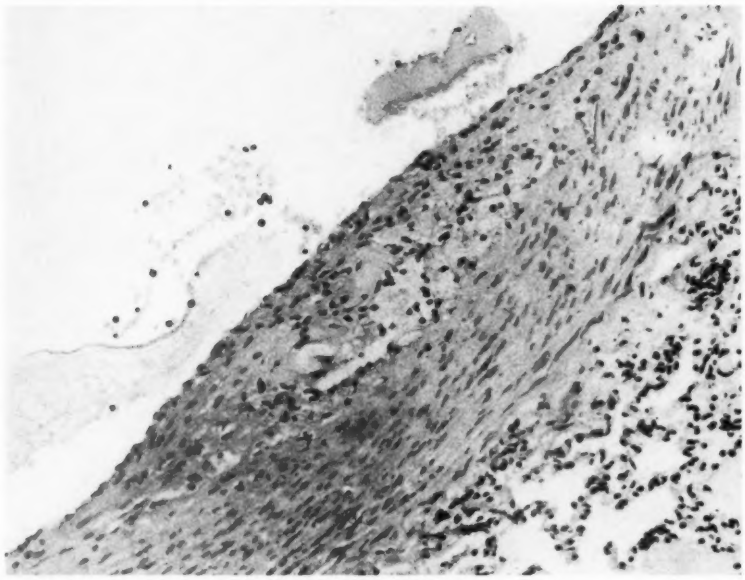

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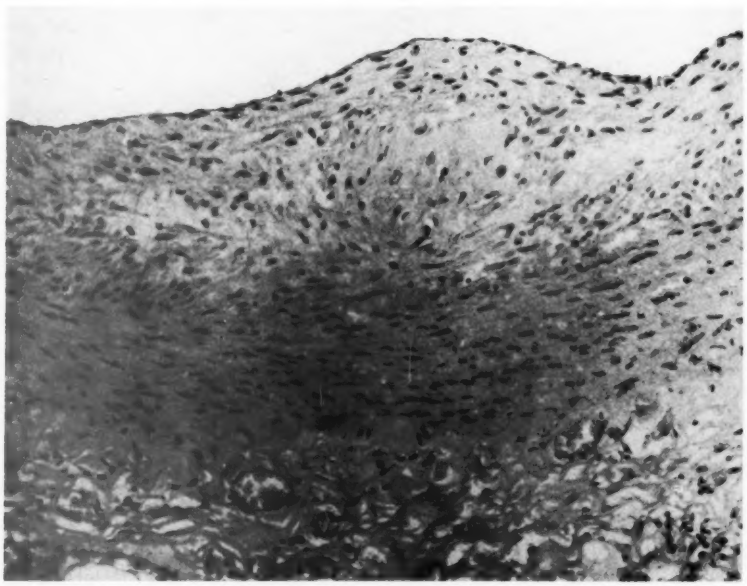

MYOCARDITIS, ARTERITIS, AND FOCAL HEPATIC, SPLENIC, AND RENAL GRANULOMAS APPARENTLY DUE TO PENICILLIN SENSITIVITY *

DOUGLAS WAUGH, M.D.†

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With increasing use of new pharmaceuticals, a variety of untoward effects may be recognized. Those due to primary toxicity are often readily apparent, but lesions due to hypersensitivity may escape notice because of the obscure relation to drug therapy. It has now been established that granulomatous lesions of myocardium, liver, spleen, kidneys, and blood vessels may result from sensitivity to sulfonamide drugs.[1-4] Clinical reactions of hypersensitivity to antibiotic agents also are well known,[5] but thus far visceral allergic lesions of sufficient severity to cause death have not been reported.

A patient in the University of Alberta Hospital developed clinical hypersensitivity to penicillin and died suddenly a short time thereafter. At necropsy the principal findings were those of diffuse interstitial myocarditis, acute and subacute arteritis, and granulomatous lesions in liver, spleen, and kidneys, similar to those seen in cases of sulfonamide sensitivity.[2]

CLINICAL OBSERVATIONS

W. R., a white male, 60 years old, was admitted to the University of Alberta Hospital on January 23, 1950, apparently in an exacerbation of cardiac neurasthenia which had been present since military service in World War I. Physical and laboratory examinations disclosed no evidence of organic disease. Symptomatic improvement followed empiric therapy with 10 units daily of crystalline zinc insulin. The patient was discharged 11 days after admission.

Three months later he was re-admitted complaining of anorexia, weakness, fatigue, and weight loss of 3 weeks' duration. Intermittent severe chest pain and cough had been present for 3 days. Past history revealed only the previously noted "cardiac neurosis." On physical examination the pulse rate was 102 per minute; temperature, 99° F.; respirations, 22 per minute; blood pressure, 152/80 mm. of Hg. There was a faint apical systolic heart murmur, and physical signs of consolidation in the upper lobe of the right lung. Electrocardiographic tracings demonstrated slight left axis deviation, tall P waves in lead 2, and low amplitude of S_3. The patient had a moderate hypochromic anemia with the hemoglobin 9.2 gm. per 100 cc., and a leukocytosis of 14,600 with 65 per cent neutrophils; the sedimentation rate was elevated to 28 mm. in 1 hour. Roentgenologic examination showed evidence of pneumonia in the right upper lobe. Culture of the small amount of whitish mucoid sputum grew only a few pneumococci. Acid-fast organisms were not found by sputum smear, culture, or guinea-pig inoculation. Blood non-protein nitrogen was 21.5 mg. per 100 cc.; serum calcium, 11.0 mg. per 100 cc. or 5.5 mEq. per l.; serum protein, 6.3 gm. per 100 cc., with albumin 3.7 gm. and globulin 2.6 gm. per 100 cc. Alkaline phosphatase

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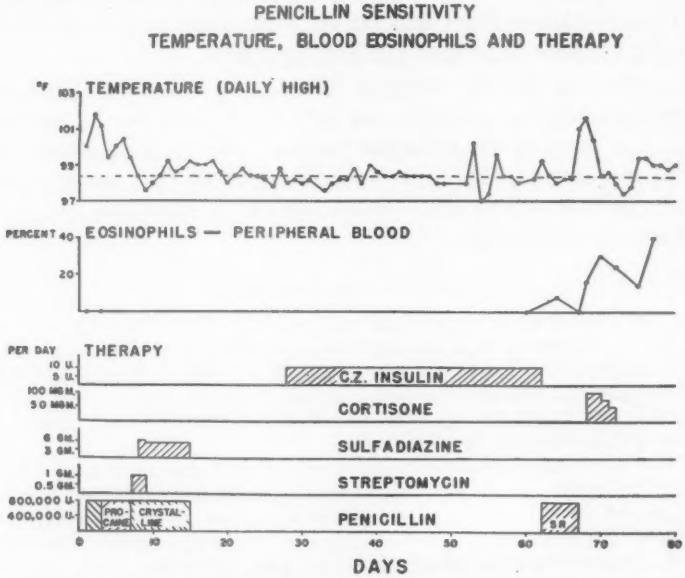
was 8.80 Shinawara units. Cerebrospinal fluid was within normal limits as to content of protein, chloride, and glucose, the colloidal gold curve was normal, and Wassermann reactions of the spinal fluid and blood were negative. A Paul-Bunnell test showed no increase in heterophil agglutinins.

Throughout an 80 day stay in the hospital, no firm clinical diagnosis was established. The consolidation in the right upper lobe failed to resolve on penicillin therapy, and the possibility of bronchogenic carcinoma was entertained. Bronchoscopy, sputum smears, and lipiodol bronchogram yielded no support for this diagnosis. Multiple myeloma was suggested by an area of rarefaction seen roentgenologically in the body of the 5th lumbar vertebra, but repeated bone marrow biopsies showed normal distribution of myeloid elements, and Bence Jones proteinuria was persistently absent.

Failing definite diagnosis, treatment was symptomatic (Text-fig. 1). Intramuscular penicillin (800,000 units per day) was administered for the first 14 days of



Text-figure 1. Graphic representation of therapy, eosinophil counts of the blood, and daily temperature during 80 days of hospitalization.

hospitalization and streptomycin was given on the 7th and 8th days. Sulfadiazine therapy was employed between the 8th and 15th days. Between the 27th and 62nd days, the only therapy was 10 units of crystalline zinc insulin per day.

Following admission the fever gradually subsided, the temperature reaching normal on the 12th hospital day. It remained under 99° F. until the 53rd day when a transient rise to 100.2° F. followed lumbar puncture. An intermittent pyrexia of low order persisted from that time until the patient died on the 80th day in the hospital. On the 61st day, he complained of sore throat and a petechial skin rash was noted on the lower legs and arms. Capillary fragility test was positive and throat culture grew *Staphylococcus aureus* (hemolytic). Intramuscular S-R penicillin* therapy (800,000 units per day) was initiated the following day. A few hours after

* Parke, Davis & Co., Detroit 32, Mich.

the first penicillin injection the skin rash became erythematous and diffuse. Skin changes became so severe that progression to exfoliation was considered to be imminent. All drug therapy was stopped on the 67th day and subcutaneous cortisone was given from the 68th to 72nd days. While the patient was on cortisone therapy the skin rash cleared rapidly and had all but disappeared by the 74th day, when edema of the ankles and shins was first noted. His pulse at that time was 96 and regular; blood pressure, 190/105 mm. of Hg. Electrocardiographic tracings showed no significant changes from those taken at admission. The patient's physical and mental condition continued to improve, but he died suddenly during his sleep on the 80th day after admission.

Blood examinations during the last 3 weeks showed leukocytosis of 15,000 to 19,000 per cmm., with progressive and marked eosinophilia (Text-fig. 1). The latter did not respond to cortisone therapy.

NECROPSY FINDINGS

Necropsy was performed 4 hours post mortem.

Residual crusted dermatitis was present on the face, axillae, and scrotum. The *lungs* were moderately increased in weight, the left weighing 500 gm. and the right 660 gm. Thin fibrous scars were present at both apices. The only other gross abnormality was the dissemination of indefinite, whitish, firm, parabronchial nodules up to 5 mm. in diameter in the lung parenchyma. The *heart* weighed 550 gm. and was diffusely enlarged and flabby. Both epicardium and endocardium were mottled with yellow-white, fairly discrete firm nodules up to 0.5 cm. in diameter. On section these were poorly circumscribed and extended into the myocardium. The lesions were most numerous on the papillary muscles. The coronary arteries were calcified but patent throughout. No other gross abnormality of the heart was seen. The *spleen* was slightly enlarged, weighing 290 gm., and showed gross changes of congestion only. The *liver* was in a state of moderately advanced chronic passive congestion, and serosal congestion was seen in the distal one-third of the appendix. Apart from scattered arteriosclerotic cortical scars, the *kidneys* were normal grossly. The *brain* weighed 1700 gm. and was not grossly altered.

Blocks of tissue were fixed in 10 per cent formalin, embedded in paraffin, and microscopic sections cut at 5 to 8 μ. Sections were stained with hematoxylin and eosin, phosphotungstic acid hematoxylin, Gram's stain, Fite's stain for acid-fast organisms, Verhoeff's elastic tissue stain, and with toluidine blue.

Microscopic Examination

Microscopic study revealed a diffuse interstitial myocarditis, disseminated acute and subacute arteritis, and focal granulomatous lesions, in liver, kidney, and spleen. Localized interstitial granulomas were present close to blood vessels throughout both lungs.

Heart. The white nodular areas noted grossly in the myocardium were highly cellular granulomas. Typically, these consisted of a small central focus of necrotic myocardial fibers, usually close to a small artery (Fig. 1). Surrounding these necrotic fibers was a relatively broad zone of cellular exudate made up mainly of eosinophilic leukocytes. Scattered among these were lymphocytes, a few myocardial histiocytes, and plasma cells (Fig. 2). At the periphery of this zone of infiltration early fibroblastic proliferation and capillary budding were present. In the same area multinucleated giant cells were numerous. These contained from ten to twenty-five nuclei and had copious acidophilic or amphophilic cytoplasm. Stained with Mallory's phosphotungstic acid hematoxylin, the cytoplasm of these cells showed the fine fibrillar structure of myoplasm. Throughout the heart, structural elements were widely separated by edema. Although the inflammatory infiltrate frequently involved the adventitia of small arteries, thrombosis was not noted.

Lung. A small, recent infarct was present in the left lower lobe of the lung. Other sections of both lungs showed patchy, poorly circumscribed areas of consolidation (Figs. 3 and 4). These consisted of accumulations of lipid-filled macrophages in alveoli, together with a granular acidophilic material. Alveolar walls in these areas showed a striking nodular thickening, often bulging into the alveolus. The nodules consisted of loosely arranged fibroblasts, eosinophils, and lymphocytes, and often contained a single multinucleate giant cell (Fig. 4). These granulomas were often situated in the adventitia of a small artery in which there was intense subintimal edema.

Spleen. In addition to intense passive hyperemia, giant cell granulomas were seen in the walls of splenic arterioles, as well as in trabeculae and capsule (Figs. 5 and 6). These foci were generally similar to those in the lung, but had a somewhat more compact structure.

Liver. Interstitial granulomas, lacking necrosis, were seen throughout the liver in close relation to portal areas (Fig. 7). These showed edema and infiltration with eosinophils and lymphocytes together with early fibroblastic proliferation. Neither giant cells nor vascular lesions were seen in the liver.

Kidney. Focal areas of cellular infiltration were seen in the adventitia of the small renal arteries. One artery (Figs. 8 and 9) showed subintimal edema and early fibroblastic proliferation, together with adventitial cellular infiltration similar to that seen elsewhere.

Arteries. In addition to arterial lesions already noted, small arteries in the pancreas and appendix showed adventitial infiltration, medial

disintegration, and subintimal edema. In the appendix there was typical fibrinoid necrosis of arterial media (Fig. 10).

All sections were carefully examined for bacteria, including acid-fast organisms, but none was demonstrated. Toluidine blue stains did not show significant amounts of metachromatic material in the lesions.

Discussion

In the case reported here, there is ample indirect evidence of sensitivity to penicillin. Prior to the second course of treatment, eosinophilic leukocytes were present in the blood in normal numbers. During therapy there was a rapid and marked eosinophilic leukocytosis, coinciding with the appearance of an erythematous skin eruption. At the time that these manifestations made their appearance, penicillin was the only drug being given other than insulin. Since insulin therapy had been continuous, it is unlikely to have been the cause of such a reaction of hypersensitivity.

In addition to these clinical phenomena of allergy, the visceral lesions were of the type seen in other hypersensitive states.[1,2,6] The arterial lesions were indistinguishable from those of periarteritis nodosa in either its acute or healing phases. The most characteristic acute lesion was seen in the appendix where fibrinoid necrosis of the media was prominent (Fig. 10). In other vessels subintimal edema and adventitial infiltration were dominant features. The vascular lesions appear to correspond precisely with those described by Rich[3,4] and by More *et al.*[2] in cases of sulfonamide allergy. The latter authors also recorded focal granulomatous lesions in splenic trabeculae, liver, and kidney similar to those seen in the present case.

Of special interest is the intense interstitial myocarditis which is assumed to have caused death. This lesion could not be explained on the basis of focal obliterative arteritis which, though sought, was not found. The zones of myocardial necrosis at the centers of these focal infiltrations appeared to be related to the intensity of the inflammatory reaction rather than to vascular occlusion. Furthermore, the character of the cellular exudate, composed predominantly of eosinophilic leukocytes, differed strikingly from that seen in ordinary cases of myocardial infarction. Fibroblastic and capillary proliferative activity in the myocardium were of a degree consistent with the clinical duration of the hypersensitive state (15 to 18 days) and with the intensity of the inflammatory changes. The ankle edema which was noted after termination of the second course of penicillin may have been a manifestation of either cardiac failure or of the increased capillary fragility

of which there was clinical evidence. It is most probable that some cardiac damage was present at this time, although this was not apparent in electrocardiograph tracings.

The pulmonary lesions present something of a problem. It is clear from the clinical history that some respiratory disorder antedated both courses of penicillin therapy, and that this, whatever its nature, did not respond to antibiotic treatment. While it might be assumed from this that the extra-pulmonary lesions also were present prior to penicillin therapy, there is circumstantial evidence that this was not so. First, there is the striking clinical allergic reaction to the second course of penicillin, together with the remarkable blood eosinophilia at that time. Second, the character of the lesions themselves, in which edema and inflammatory infiltration predominate rather than fibrosis and scarring, is that of young lesions, weeks, rather than months, old.

If these assumptions are acceptable, it can then be suggested that the sequence of events was roughly as follows: (1) Establishment of a subacute pneumonitis of unknown type, (2) treatment (and sensitization) with the first course of penicillin, (3) fatal disseminated allergic tissue reactions induced by the second course of penicillin.

Even casual perusal of published reports provides a number of parallel, if not identical, cases. In 10 of 22 cases of sulfonamide allergy reported by More *et al.*,[2] the reason for therapy was a respiratory infection. In the case of diffuse eosinophilic arteritis reported by Lumb,[7] chronic cough was the presenting complaint, although the question of antibiotic therapy was not dealt with. Churg and Strauss[6] have recently recorded 13 cases of bronchial asthma, hyper-eosinophilia, and widespread granulomatous vascular lesions similar to those in the present case. Although drug sensitivity was not recorded in their cases, nearly every one had recurrent episodes of pneumonia, and at least some of these may be presumed to have been exposed to drug therapy, perhaps repeatedly.

It seems unwisely speculative to theorize further than to point out the parallel or antecedent development of respiratory disease in those cases in which the patients ultimately died with cardiovascular granulomas of such close anatomical similarity. In the light of the apparent pathogenetic significance of penicillin sensitivity in the case reported here, however, it can be suggested that drug allergy in the presence of respiratory disease may manifest itself in granulomatous lesions of this type. It can further be postulated, after comparison of the findings in this case with those of others,[1,2,6,7] that the lesions do not appear to be specific and recognizable as to causative agent. Indeed, it

is clear that prior to the present concept of allergic diseases, these cases would have been recorded simply as instances of periarteritis nodosa, with or without unusual features.

It is still impossible to say categorically that periarteritis is always a specific lesion of hypersensitivity. However, as cases of this type increase in number and in frequency of recognition, a body of evidence may be assembled upon which to base more detailed and precise studies of pathogenesis. At the present time, it seems wiser to contribute factual data on these cases, rather than to use them as a foundation for structurally insecure hypotheses.

Summary

In a case in which death apparently resulted from visceral lesions of penicillin sensitivity, necropsy findings included lesions of periarteritis nodosa, and hepatic, splenic, and renal granulomas, as well as an intense interstitial myocarditis. The lesions were similar to those seen in sulfonamide sensitivity. There is a possibility that chronic pulmonary disease of unknown type may act as a conditioning factor in the development of tissue lesions of hypersensitivity.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

All sections were fixed in 10 per cent formalin and stained with hematoxylin and eosin.

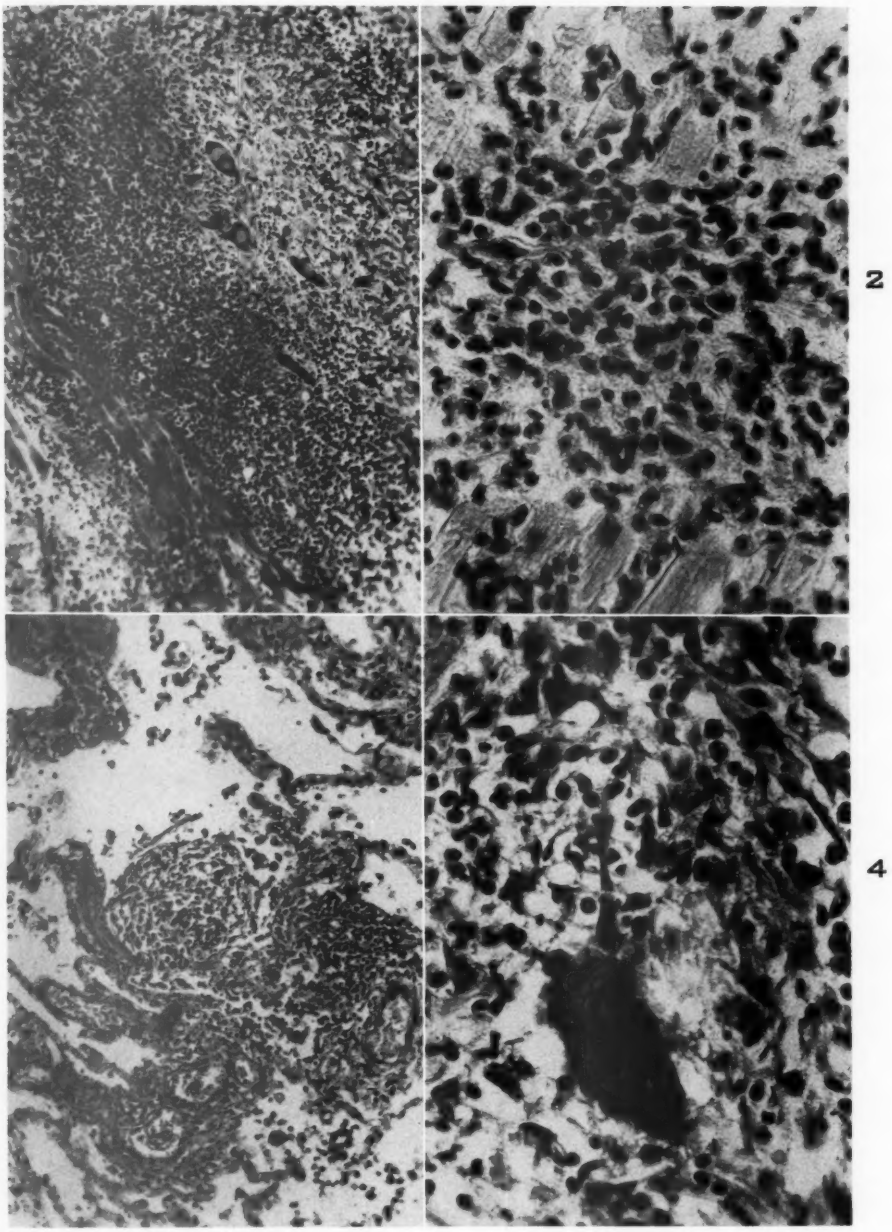
PLATE 72

FIG. 1. Margin of myocardial granuloma showing giant cells and heavy cellular infiltrate. Alignment of giant cells parallel to muscle fibers may be noted. × 315.

FIG. 2. Cellular infiltrate in myocardium, consisting mainly of eosinophilic leukocytes. × 645.

FIG. 3. Lung. Paravascular granuloma bulging into alveolar lumen. Cellular infiltrate consists mainly of mononuclear cells, with sprinkling of eosinophils. × 150.

FIG. 4. Lung. Giant cell in the center of a paravascular granuloma. × 645.



Waugh Granulomas Due to Penicillin Sensitivity

PLATE 73

FIG. 5. Spleen. Paravascular granuloma with prominent giant cell reaction. × 150.

FIG. 6. Spleen. Similar to Figure 5, but at a higher magnification. × 645.

FIG. 7. Liver. Localized interstitial infiltration consisting mainly of lymphocytes with a few eosinophils. × 315.

FIG. 8. Kidney. Subacute arteritis with heavy lymphocytic and eosinophilic infiltrate in the adventitia. × 315.

FIG. 9. Kidney. Small focus of fibrinoid alteration in adventitia of artery shown in Figure 8. × 645.

FIG. 10. Appendix. Acute arteritis with fibrinoid alteration of media and inner adventitia. × 315.

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7

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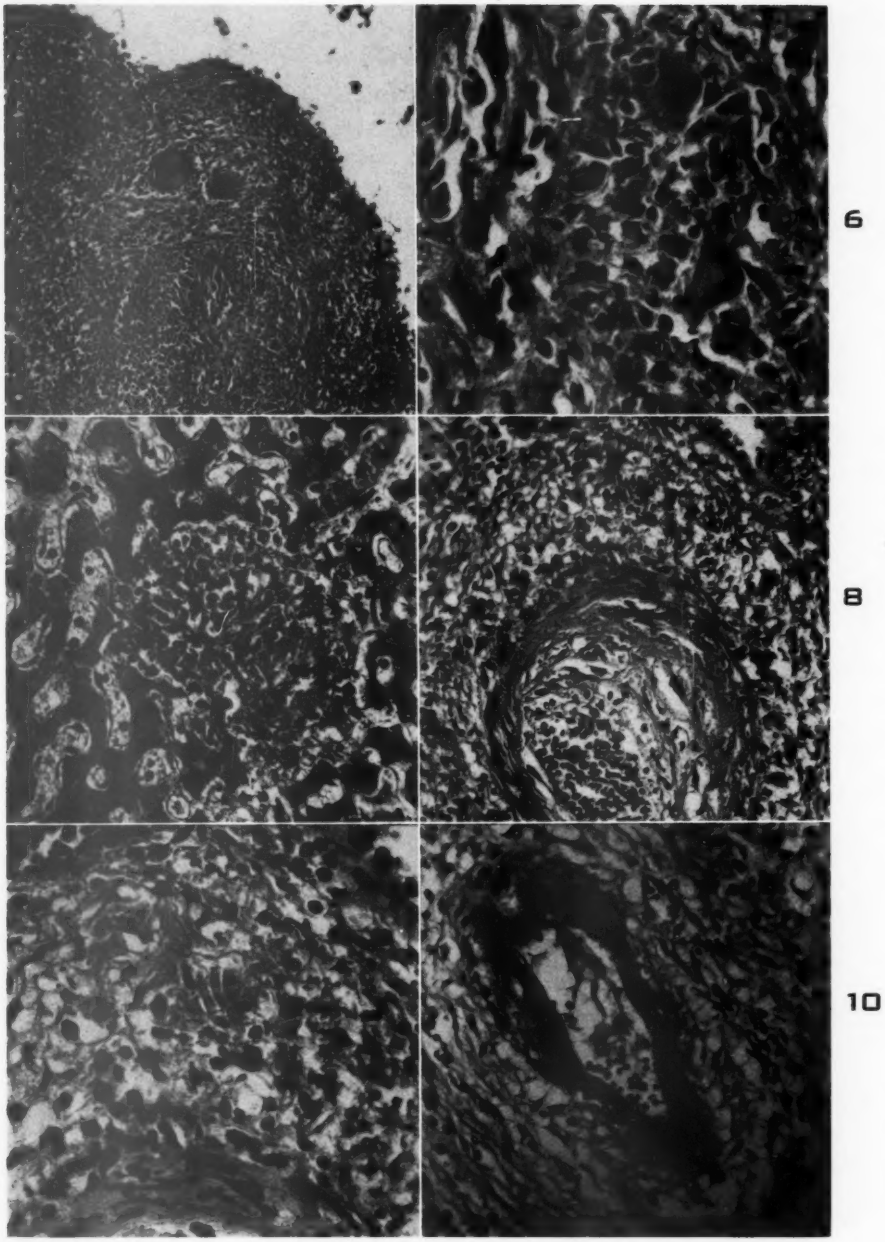

Waugh Granulomas Due to Penicillin Sensitivity

THE NATURE OF AGENESIS AND DEFICIENCY OF PARTS

The Influence of Intrinsic Factors in Disease When Development of the Body Is Abnormal, as Illustrated by Agenesis of the Digits, Facial Hemiatrophy, and Cerebral Agyria and Microgyria *

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In this paper the terms intrinsic factor and intrinsic factors include neural intrinsic factor and basic intrinsic factor. In an earlier paper[1] I defined neural intrinsic factor as of intrinsic nature and of neural origin, related to specific nerve sheath tissue (neurilemma or sheath of Schwann). Basic intrinsic factor was defined as more primitive and comprehensive than neural intrinsic factor and as operating from the time of fertilization of the ovum. Basic intrinsic factor is regarded as embracing a potential neural intrinsic component.

According to the hypothesis advanced in this paper, agenesis of a part of the body, that is the failure of such a part to develop completely, may be due to either intrinsic factors or extrinsic factors exercising their influence on various parts of the embryo at an early stage of its development, and it may be impossible from a study of the end result alone to say whether intrinsic factors or extrinsic factors were responsible for the abnormality. The end result does not show an exact replica of what was present at the time the disturbance in the embryo occurred; growth and some differentiation take place but they fall short of normal growth and development, and the term static may reasonably be applied to these lesions.

According to the present hypothesis, intrinsic factors are capable not only of influencing the production of such static or stationary lesions but also of acting at intervals or continuously before and/or after birth and thus of influencing the production of lesions which are dynamic and progressive. The extent (if any) to which intrinsic factors can influence the production of such dynamic or progressive lesions is doubtful.

Dynamic or progressive lesions, due to the influence of intrinsic factors, may be characterized by increase or diminution of bulk. In an earlier paper[2] I was concerned with such lesions showing increased bulk; the present paper is concerned with such lesions showing diminished bulk. According to the present hypothesis the same intrinsic factor acting differently (either in manner or degree) may produce

* Received for publication, August 29, 1951.

different, and indeed apparently opposite, effects. Sometimes both effects are to be seen in the one individual as in case 1.

Agenesis of Digits

A group of cases will now be described in which defects of digits are a conspicuous feature. Attention will be drawn also to a variety of pathologic conditions with which digital defects may be associated.

Case 1 *

The patient was a youth, 19 years old. He had a nevus on the left cheek. In Figure 1 the left index finger and thumb are seen to be greatly enlarged. The increase of subcutaneous fat gave the feeling of a lipoma. The right forearm and hand are enlarged; the right thumb and index finger are smaller than normal, the other digits being of normal size. Macrodactyly with excess of adipose tissue has been found associated with neurofibroma[2] and is considered to be a manifestation of neurofibromatosis (von Recklinghausen's disease). Small size of bones is also sometimes associated with neurofibromatosis.[4,5]

Local deficiencies of parts of a limb-bud may be unassociated with other defects or diseases; sometimes, however, they are associated with such conditions and case 1 provides an example which may be linked with neurofibromatosis. Case 2 presents an example of defective limb-bud which may be linked with Osler-Rendu disease or hereditary hemorrhagic telangiectasia.

Case 2

The patient (S. H. 14804) was a man, 26 years of age, who had telangiectases of the skin of the face and of the mucous membrane of the lip. These were first noticed when he was 21 years old. The patient said his father had exactly the same condition, and he believed it was present in the previous generation (grandmother on the father's side). There was congenital malformation of the patient's right hand (Fig. 2).

Garland and Anning[6] described a patient with generalized telangiectasis of the face and typical von Recklinghausen's disease with pigmentation and tumors. Ito *et al.*[5] described, in a patient with abortive von Recklinghausen's disease, a small fourth metatarsal bone on each side. The small metatarsal bones were due, presumably, to developmental defects in these two limb-buds.

In keeping with the hypothesis already presented[1] that intrinsic factor underlies both neurilemmoblastosis (tuberous sclerosis com-

* Taken, with permission of the Carnegie Institute of Washington, from the paper by Dr. George L. Streeter.[3]

plex) and neurofibromatosis, it is suggested, in view of the associations mentioned, that intrinsic factor also underlies hereditary hemorrhagic telangiectasia, and that in case 2 both the malformation of the hand (due to developmental defect of a limb-bud) and the condition of telangiectasia are linked etiologically at the basic intrinsic factor level.

A case will now be described in which all four limbs showed developmental defects.

Case 3

The patient (P. U. S. 6170) was a woman, 53 years of age, who had suffered from bleeding from the rectum for 8 years. She was only 140 cm. in height, and her mental faculties were below normal. Her hands and feet showed congenital abnormalities, portions of fingers and toes being absent (Figs. 3 and 4). A snipping was taken from the rectum 20 cm. from the anus. On histologic examination inflammatory changes and some hyperplasia of the glandular elements were found, but no convincing evidence of neoplasia. The points of interest about this case are the involvement of all limb-buds, the short stature, and the subnormal mentality.

Microdactyly and macrodactyly may occur in the same patient as in case 1, and since important pathologic changes, including neurofibroma, osteochondroma, and lipoma, may occur in relation to macrodactyly (Bell and Inglis,[7] and Inglis[2]) the possibility of pathologic conditions being associated with microdactyly seemed worth investigating. With this in mind a histologic study was made of the abnormal part of the foot in case 4.

Case 4

The patient (P. U. S. 6974) was a male infant, 3 months old. The limbs were equal in length but the toes of the left foot, except the great toe, were webbed and considerably smaller than normal (Fig. 5). Both hands were normal. The thymus weighed 38 gm. and covered the whole of the upper mediastinum. Otherwise the necropsy revealed no changes of special significance.

A transverse section was made through the left foot where the four outer toes were small; blocks were taken including the whole width of the foot above and below the transverse section. The serial sections which were cut proximally and distally to the plane of section through the foot revealed no changes of significance.

In cases 1 to 4 the small size of digits or the absence of digits is thought to be primary, and due to local developmental deficiencies; sometimes, however, small size of digits is secondary and due to faulty

innervation. Case 5 is regarded as an example of the secondary type, and is included for contrast.

Case 5

The patient was a man, 34 years of age, whose right leg was amputated. This case corresponds to case 1 in the paper by Jackson[8] on familial lumbo-sacral syringomyelia. Figure 6 shows the great shortening of the right foot and of its toes; the other foot was similar. These changes are secondary, and are considered to be due to faulty innervation. A large ulcer was present on the ball of each foot. These ulcers probably were due to faulty innervation, whereas the ulcers which appear to be present on the soles of the feet in case 3 (Fig. 4) are thought to be due to local influences in the affected parts.

NATURE AND SIGNIFICANCE OF FOCAL DEFICIENCIES OF THE LIMB-BUDS

Anatomical defects may be due to extrinsic factors or to intrinsic factors. Mall[9] said that monsters are produced from normal embryos by influences which are to be sought in their environment. Extrinsic factors are in play when anatomical defects are due, for example, to infection (Gregg[10]), faulty nutrition (Warkany, Nelson, and Schraffenberger[11]), or x-rays (Bagg[12]). Stockard,[13] who carried out much experimental work using extrinsic factors when treating embryos in various ways, said that the primary action of all treatments is to inhibit the rate of development and that the type of deformity that results depends simply upon the developmental moment at which the interruption occurs.

In regard to anatomical defects due to the influence of intrinsic factors, the work of Streeter[8] is of outstanding importance. He showed that intra-uterine amputation of portions of limbs or of digits is due to focal deficiencies in fetal tissues and not to constriction by amniotic bands or adhesions. He showed how circumscribed areas of limb-bud tissue, due to an exceptional growth vigor, may develop to twice their normal size. He also showed the opposite in cases in which, because of extreme deficiency, a given part is entirely absent, there being no evidence that even its primordium was ever present. Intermediate between these two is that in which a given part, although normal enough in size, is aberrant in form. Streeter pointed out that the digits supply the most numerous representatives of this group, through their marked disposition to such deformities as doubleness, the different types of extra fingers, webbed fingers, short fingers, stunted fingers, and anomalous joints. Streeter emphasized that these lesions are primarily involved in the constitution of the germ plasm.

When discussing the abnormalities in the right hand of a 5-months old infant (Tulane, no. 5605), Streeter[3] said: "It is of importance to note that the third digit, which has a groove on the palmar surface of its distal segment, is not undergoing normal development, in spite of the fact that the groove only partially encircles it . . . The stunting of this distal segment is plainly not a result of the groove but rather a product of the same condition that produced the groove." In this digit both agenesis and incipient amputation are present.

Though Streeter[3] spoke of the creases as deficiency creases and did not refer to them as cicatricial (at all events in the early stages), he did say, when referring to the left arm of a child of 2½ years (no. 5493), that above the wrist was a definite crease that encircled the arm. "This crease has flattened out somewhat in the two and one-half years intervening between the pictures. At the bottom of the furrow the skin appears to have a different character resembling a scar."

In Streeter's[3] cases there were annular rings elsewhere than around digits, and it would seem from the photographs that in some situations fibrosis was present not merely as linear creases, but as fairly extensive areas involving the skin and probably the underlying soft tissues; such changes seem to be evident in Streeter's Figures 46 (involving skin of chest wall of fetus) and 89 (involving skin of leg of a girl, 17 years of age).

In Streeter's article[3] there is a photograph of a family, four of whose members, in two generations, exhibited agenesis of hands and feet; the phenomenon, he said, was evidently hereditary.

Keith[14] expressed the view that the focal deficiencies in fetal tissues described by Streeter[3] were due to breakdown in the circulatory system of the fetus, but this would not account for several of the features of Streeter's cases; in particular it would not account for the creases partially or completely surrounding a limb or digit, nor would it account for the occurrence of overgrowth and undergrowth of different parts of one limb.

RELATION OF ANNULAR CREASES AND CONSTRICTING BANDS ASSOCIATED WITH AGENESIS OF DIGITS, TO AINHUM, SCLERODERMA, LOCAL PANATROPHY (GOWERS), FACIAL HEMIATROPHY, RAYNAUD'S DISEASE, ACROCYANOSIS, DYSPLASIA OF NAILS, AND MELORHEOSTOSIS

Streeter[3] said that since the end stages of ainhum bear a striking resemblance to some of the later phases of intra-uterine amputations, one wonders if they may not be related processes. The pathology of ainhum was described by Unna[15] as a primary degeneration of the

skin, a sort of ring-formed scleroderma. As a result of the present study it is suggested that the annular rings, grooves, and diffuse scars described by Streeter[3] may link up with some examples of scleroderma, and that the healed or unhealed ulcers in Streeter's cases (Streeter's Figures 67, 68, and 92) may link up with those examples of loss of tissue referred to as local panatrophy of Gowers (Gowers,[16] Bettley[17]).

Scleroderma has occurred in patients with melorheostosis (Hall,[18] Gillespie and Siegling,[19] Dillehunt and Chuinard[20]). Melorheostosis has occurred in patients with neurofibromatosis (McCarroll[21]) and in a patient with tuberous sclerosis (Hall[18]). It has been suggested[1] that intrinsic factor influences the development of neurofibromatosis and of neurilemmoblastosis (tuberous sclerosis complex), and it is now suggested, in view of the associations mentioned, that intrinsic factor may influence the development of melorheostosis and of scleroderma.

Lewis and Landis[22] said that the combination of scleroderma and Raynaud's disease occurs too frequently to be fortuitous, and that the changes in the skin are occasionally accompanied by curious hemiatrophic changes in face or tongue. Lewis[23] found from his experiments that the defective circulation in Raynaud's disease is not nervous in origin, but is due to spasm of the digital arteries due to a peculiar condition of the vessel wall locally. Lewis and Landis[24] stated that the essential disturbance in acrocyanosis is obstruction in the cutaneous arterioles. The spasm is not increased vasomotor tone; its cause is to be sought in the vessels themselves.

Hiller[25] described a woman, 36 years of age, who, since birth, had exhibited bands of constriction of the skin above the right wrist, at the proximal ends of the four outer fingers of the right hand, immediately distal to the right knee joint, and at the right ankle joint. Amputation of the right foot had been performed 17 years before for threatened gangrene. Three years previously, and for a similar reason, the leg was amputated at a point 7 cm. above the right knee joint. Shortly after the operation another constricting band appeared just proximal to the end of the stump. The constrictions appeared to be due to fibrous contraction limited to the subcutaneous tissue of the region, nowhere involving the muscles. On the left foot there were four toes only, the three outer being webbed. Here we have association of digital agenesis and annular cicatricial bands, but the point of outstanding importance is that not only were bands of constriction present at birth, but when the patient was 33 years of age another constricting band appeared and progressed, showing that the formation of these constricting bands is a dynamic process.

A point worth noting in one of Streeter's[3] cases is that in some digits minor deficiencies of nails appear to be present (Streeter's Figures 95, 96, and 97). As a result of the present study it is suggested that these may be akin to (a) heredo-congenital onychogryphosis, as described by Schmidt,[26] occurring in association with total alopecia and schizophrenia, (b) ungual sclerosis associated with cutaneous achromia, hyperchromia, and abnormal development of hair on parts of the skin of the body in a patient suffering from familial corticomeningeal angiomatosis and progressive demyelination of the white substance of the brain as recorded by Divry and van Bogaert,[27] (c) dysplasia of fingernails found associated with arthrodysplasia of elbow joints and rudimentary patellae described by Hawkins and Smith[28] and by Wildervanck.[29]

In view of these observations it is suggested that intrinsic factor may underlie some examples of agenesis of digits, annular creases, constricting bands, ainhum, scleroderma, local panatrophy (Gowers), facial hemiatrophy, Raynaud's disease, acrocyanosis, dysplasia of nails, and melorheostosis.

RELATION OF CONSTRICTING BANDS (AINHUM) TO HEREDITARY ICTHYOSIS OF PALMS AND SOLES, AND TO SYRINGOMYELIA

Hereditary icthyosis of the palms and soles is a well recognized dermatologic condition. Bramwell[30] recorded a typical example. The condition, practically speaking, is limited to the palms and soles. Bramwell's photographs show no suggestion of constricting bands of toes or fingers. Vohwinkel[31] described a case of hereditary keratoma of palms of hands and soles of the feet. The hands showed the following mutilations: two phalanges were missing from the right index finger and from the right little finger and also from the left little finger. The distal part of the left index finger was in process of separation. He believed that the amputations were due to strangulation caused by the process of keratinization.

Wigley[32] recorded a case of hyperkeratosis palmaris et plantaris associated with ainhum-like constrictions of the fingers. He said that the condition of the fingers resembled that of sclerodactylia, a variety of scleroderma. The distal phalanx of the little finger of the left hand was missing, and around the center of the middle phalanx of the corresponding finger of the right hand was a constricting band of thickened, hard skin. This band subsequently caused spontaneous amputation of the finger exactly as in the left hand (compare Tatz's[33] case). Wigley said that nearly all cases given the name ainhum have occurred in colored patients, but Streeter[3] described and illustrated (Streeter's

Figures 89 to 92) grooves and encircling creases in the hands and legs of a white girl, 17 years old, without mentioning palmar and plantar keratoses.

Tatz[33] described a patient with keratoderma of palms and soles. The thumb and distal halves of the fingers of the right hand had deep ainhum-like constrictions encircling them. Neurologic examination revealed typical signs of syringomyelia. The changes in the hands preceded the syringomyelia by many years. Six years before examination a fissure at the base of the left thumb deepened to such an extent that the thumb fell off (compare Wigley's[32] case).

It will be seen from this study (1) that keratosis palmaris et plantaris may occur alone, and without encircling bands or amputations (Bramwell[30]), (2) that encircling bands leading to amputation of digits may occur without keratosis palmaris et plantaris, and without syringomyelia, and in white as well as in colored people. It is therefore suggested that though encircling bands and amputations are caused neither by syringomyelia nor by keratosis palmaris et plantaris, the fact that these uncommon conditions occasionally occur in the same patients suggests that they may be etiologically related, and is in keeping with the opinion that intrinsic factor, probably at the basic level, may underlie them all.

RELATION OF LIMB-BUD DEFECTS TO LENTIGINES

Ito *et al.*[5] described a patient with abortive (pigmentary) von Recklinghausen's disease whose skin was studded with lentiginous pigmented spots. Roentgenologic examination showed both fourth metatarsal bones to be of small size. I[4] submitted evidence in support of the opinion that small size of individual bones and neurofibromatosis are linked at the basic intrinsic factor level. It is therefore suggested that in the case of Ito *et al.* a similar link exists between the small size of the fourth metatarsal bones and the lentiginous pigmented spots. It consequently seems worth while to ascertain what lesions may accompany, and possibly be related to, lentigines, and in that regard the following case is of interest.

Case 6

The patient (P. U. S. 6994) was a woman, 31 years of age. She had three sisters and two brothers, none of whom showed any congenital defect. Very soon after birth there appeared on the patient's skin thousands of small "freckles." Most of these were brown, but many were black. They were not related to exposure to the sun. Indeed, the face was less thickly sprinkled than most other parts of the body

(Figs. 7 and 8). They were considered by dermatologists, on clinical grounds, to be "lentigines." The patient was congenitally deaf.

Two features call for comment: the lentigines and the congenital deafness.

Lentigines. Lever[34] said that histologically lentigo represents an early junctional nevus in which the process has not progressed to the stage at which dropping off of nevus cells into the corium occurs. All steps in transition to the junctional nevus may be encountered. Wohlwill[35] published an account of a patient (his case 3) with coloboma of the upper eyelid, dermoids of the conjunctiva, fibromata pendula of the tragus, absence of corpus callosum, partial absence of the falx durae matris, arterial angioma of the trigonum, and multiple pigmented nevi of the skin. Leschke[36] described under the heading "dystrophia pigmentosa" a syndrome in which hundreds of small nevi "like those of von Recklinghausen's disease" were present in the skin and there were signs of disturbance of endocrine glands; in particular, a variation of dystrophia adiposo-genitalis. In discussing Leschke's paper, Weber described a man, 40 years of age, whose whole body was covered with small, blackish, pigmented nevi (lentigines) but who had not the typical dystrophia adiposo-genitalis mentioned by Leschke. Jeghers, McKusick, and Katz[37] described in 10 cases, most of which were heredofamilial, melanotic spots of the oral mucosa, lips, and digits in patients with generalized intestinal polyposis, especially conspicuous in the small intestine.

In keeping with the hypothesis advanced in this paper, it is suggested that intrinsic factor at the neural or basic level may influence the development of lentigines, defects of limb-buds, the abnormalities associated with lentigines in Wohlwill's[35] case 3, dystrophia adiposogenitalis, and generalized intestinal polyposis.

Congenital Deafness. Mayer[38] reported his pathologic findings in 13 cases of congenital deaf-mutism and 2 cases of hereditary hardness of hearing with malformations in the inner ear. Of these, 3 were associated with otosclerotic foci and 2 with bony changes resembling otosclerosis. Fraser[39] and others have reported otosclerosis and fragilitas ossium (osteogenesis imperfecta) occurring in the same individual. Fairbank[40] described osteogenesis imperfecta (his case 2) in a patient with bending of the tibia very like that often considered to be related to pseudarthrosis. Wade[41] found pseudarthrosis of the tibia in one of three siblings. The second was a deaf-mute and the third suffered from congenital pyloric stenosis. Crooks[42] reported congenital hypertrophic pyloric stenosis in a girl, 7 weeks of age, who had osteogenesis imperfecta. Wyburn-Mason[43] referred to a case of otosclerosis with

arteriovenous angioma of the spinal cord and segmental pigmented skin nevus. Ito *et al.*[5] recorded small cutaneous pigmented areas (lentigines) in a case of abortive von Recklinghausen's disease with developmental defects of limb-buds (both fourth metatarsal bones). Love and Addison[44] referred to a boy with developmental defects of limb-buds (deficient digits of the right foot and both hands), who was born deaf. Bell[45] and Dax[46] described limb-bud defects (polydactyly) in patients with retinitis pigmentosa. Bell found, in 919 cases of retinitis pigmentosa, 96 cases of deaf-mutism. Wyburn-Mason[47] found pigmentary degeneration of the retina in a patient (his case 44) with von Recklinghausen's disease. Preiser and Davenport[48] found neurofibromatosis (von Recklinghausen's disease) in the sibling of a patient with Hirschsprung's disease. Lockhart-Mummery[49] found, in 100 cases of congenital dilatation and hypertrophy of the colon, 2 deaf-mutes.

Because of the associations mentioned, it is suggested that intrinsic factor may underlie some examples of deaf-mutism, otosclerosis, osteogenesis imperfecta, pseudarthrosis of the tibia, congenital pyloric stenosis, cutaneous lentigines, polydactyly, retinitis pigmentosa, von Recklinghausen's disease (neurofibromatosis), and Hirschsprung's disease. In particular it is suggested that in case 6 the cutaneous lentigines and the congenital deafness have a common etiologic background, intrinsic factor having influenced the development of both.

Facial Hemiatrophy

Wartenberg[50] quoted observers who considered that progressive facial hemiatrophy has intimate connections with scleroderma. He spoke of it as a heredo-degeneration and said that many facts indicate that it might be a pathologic condition which belongs to the vast group of developmental defects so abundant in neurology. He added that the course of hemiatrophy is the same as that of all other heredo-degenerations: it begins in pre-adolescence without apparent cause; it develops slowly and later becomes stationary. In some of Wartenberg's illustrations the lesions seem to be characterized by great deformity. In others, for example his case 5 and Figure 4, the changes are less marked, and the appearances somewhat resemble those of case 7 in this paper, in which, except for the tumors on the eyeballs, the condition appeared to be stationary and not progressive after birth.

Case 7

The patient (S. H. 4813) was a girl, 13 years of age, who was subnormal mentally. She previously has been described briefly by

Cohen.[51] Her face was asymmetric, the left side being smaller (Fig. 9). The lower jaw was directed to the left. The tongue had a tendency to the bifid type, and was asymmetric. The left side of the tongue was smaller and firmer in consistency than the right (Fig. 9), and the tip of the tongue was directed to the left. Movement of the tongue was restricted, especially in a forward direction. At birth the child had "double tongue-tie" and could not take the nipple until the tongue was operated on. The left eye had normal lids; the conjunctiva was bulged forward at the outer canthus by a fleshy, soft mass (Fig. 9); this had been removed 2 years before but had recurred. In the right upper lid near the outer canthus, there was an area of "hypertrophy," on the under surface of which were abnormal eyelashes. The conjunctiva at the outer canthus was bulged forward by a mass (Fig. 9) similar to that on the left eye. This growth on the right eye had previously been removed 18 months before, and had gradually recurred until it reached its former limit of extension on the cornea. This tumor mass was excised and found to be composed of dense fibrous tissue and adipose tissue. The left ear was malformed; an accessory lobule was present just in front of the tragus, and the helix was unduly curved over (Fig. 10). The left auditory meatus was imperforate. The patient had a harsh voice, indicating some affection of the larynx which may have been congenital.

The hands showed considerable deviation from normal. The digits were extraordinarily long, and very slender; each middle finger was about 1 cm. longer than those of a normal male adult. Each little finger tended to project medially; it did not run parallel with the others and was kept slightly flexed (Fig. 11); the cause of the flexion was not clear, but there was no heaping up of the skin such as is commonly seen in Dupuytren's contracture. The mother's two little fingers showed slight deviation from normal; the joint between the proximal and second phalanges was raised when the hands were placed palm downwards on a table.

There were 9 siblings; one was said to be mentally deficient, another to have two fifth toes which were "more like fingers than toes" and crossed the dorsal surfaces of the adjacent toes. This description corresponds with the appearance of the toes of a patient with arachnodactyly described by Young[52] (Young's case 1, Fig. 3).

In case 7 the condition of the tongue was present at birth and presumably the facial condition also was present at birth; neither appeared to be progressive. The facial asymmetry in case 7 differs from (but may be akin to) the progressive facial hemiatrophy, characterized by conspicuous fibrosis and deformity, which is widely re-

garded as a form of scleroderma. It seems worth noting that Martin[53] reported a case of facial hemiatrophy commencing at the age of 15 in a woman 20 years of age whose left breast had never developed, and that Simpson[54] recorded congenital absence of the breast in a woman whose left hand was smaller than the right. In keeping with the present hypothesis it is suggested that, in these 2 patients, intrinsic factor (probably at the basic level) may have influenced the development of facial hemiatrophy in one, limb-bud defect in the other, and mammary defect in both.

Warternberg,[50] discussing progressive facial hemiatrophy, said that Stief[55] found atrophy of the internal organs homolaterally, including the kidney, adrenal gland, ovary, and vocal cord (compare the harsh voice in case 7).

Phacomatoses. According to Brouwer, van der Hoeve, and Mahoney,[56] the phacomatoses include Bourneville's disease (tuberous sclerosis), von Recklinghausen's disease (neurofibromatosis), von Hippel-Lindau's disease, and Sturge-Weber's disease. Wohlwill[35] said the phacomatoses comprise not only the four classical diseases included in this category, but also other pathologic groups, some of these having nothing in common with the classical phacomatoses. An example was provided by his case 3 already referred to (see lentigines). Wohlwill's case 3 has several features in common with case 7 in this paper. According to the hypothesis advanced in this contribution, intrinsic factor, sometimes at the neural level, probably more often at the basic level, may directly or indirectly underlie all the lesions in Wohlwill's case 3, all the lesions in case 7 of the present paper, and the primary lesions of the four classical phacomatoses, whether these lesions occur singly or in various combinations and whether they are stationary or progressive.

Arachnodactyly. It would seem that arachnodactyly was present in case 7. In some of the published case reports evidence is to be found suggesting that arachnodactyly may be familial (Ingram and Inglis[57]) and possibly hereditary (Young[52]). It also has occurred in association with other conditions of doubtful etiology; for example, amyotonia congenita (Young[52]), fragilitas ossium and blue sclerotics (Ellis[58]), honeycomb lungs (Oswald and Parkinson[59]), dissecting aneurysm of the aorta (Etter and Glover[60]), retinitis pigmentosa (Dax[46]). It is to be noted that in arachnodactyly certain parts may show disproportionate enlargement; for example, elongation of the great toes (case 2 of Lambie *et al.*[61]), and increased length of the intestine (Salle[62]). One or more of the components of the arachnodactyly syndrome seem to have been present (in patient or close relative) in association with

the following conditions, even though the term arachnodactyly may not have been used in the published accounts: hereditary ataxia of the Pierre Marie type with strictly sporadic optic atrophy and with acrocyanosis (Andre-van Leeuwen and van Bogaert[63]), muscle dystrophy (Goodhart[64]), and otosclerosis (Bauer and Stein[65]).

In keeping with the hypothesis advanced in this paper, it is suggested that intrinsic factor probably at the basic level may influence the development of arachnodactyly and some examples of the associated conditions mentioned.

Cerebral Agyria and Microgyria

Developmental abnormalities of the brain may assume various forms and are sometimes associated with developmental abnormalities in other parts of the body. An example of this association will now be described.

Case 8

The patient was a girl, 1 year old, with markedly asymmetric skull, inability to extend the right knee and hip completely, but no spasticity. Skiagrams showed marked plagiocephaly, slight subluxation of the left hip and very slight of the right, and no gross changes in the knee joints. The clinical diagnosis was arthrogryposis multiplex and plagiocephaly. The child died from suffocation caused by food entering the trachea. At necropsy all organs appeared normal except the brain.

A photograph of the inferior surface of the brain is seen in Figure 12 which shows, on the right side of the cerebrum posteriorly, an extensive area in which there is little if any evidence of convolutional pattern, and, on the inferior surface of the right frontal lobe, an area of conspicuous microgyria. These two areas on incision seemed to be of normal consistency. They were examined microscopically but the preparations were unsatisfactory, probably owing to faulty fixation of the brain. As far as an opinion could be formed from the sections, they showed apparent absence of some neurons and faulty formation of others, but no striking degree of gliosis.

Two features of this case call for comment: the abnormalities of the inferior extremities and the cerebral malformation.

Abnormalities of Inferior Extremities

Middleton[66] used the name myodystrophia foetalis deformans for arthrogryposis multiplex congenita and described it as a congenital deformity affecting both upper and/or lower extremities, characterized by clubhands and clubfeet, limitation of the normal range of movement at the joints, weak and wasted muscles. When the lower extremities

are affected, congenital dislocation of the hip-joints is frequently present and in the more common cases in which the knee joints are fixed in hyperextension, congenital dislocation of the knees with absence of patellae may result. Middleton's case 3 was a female child, 6 months old, with double talipes equinovarus, knee joints in a position of hyperextension, only a trace of passive flexion being obtainable, congenital dislocation of both hips, microcephaly, and mental deficiency.

Shattock[67] found in a male fetus of about full term that the thighs did not admit of extension, and that flexion of the knees was forward. Dissection, however, showed no reversal of the parts forming the joint, which admitted of abnormal over-extension without proper flexion. There was also complete absence of the right radius, a rudimentary pedunculated thumb, and sacral spina bifida.

Kenwell[68] found in a child, who died when 31 days old, that flexion of the knee joint was impossible, motion being present only in the form of hyper-extension. There were intra-uterine fracture of the left tibia, polydactylism, absence of the mesial surface of the posterior two-thirds of the right hemisphere of the brain and entire corpus callosum, as well as many other developmental anomalies.

Greig,[69] under the title "Acrodysplasia. Type: syndactylic oxycephaly," described a woman who died at the age of 62 years. She was observed at birth to have a curiously shaped head associated with malformation of the fingers and toes. During childhood and adolescence certain restrictions of movements of hands, elbows, hips, and knees became obvious, which Gregg attributed to myodystrophy or myodysplasia and regarded as a developmental defect. He was inclined to attribute the condition to hormonal influence.

Ellis, Sheldon, and Capon[70] said that, clinically, the outstanding feature in gargoylism is the limitation of movement (principally extension) which is found at the shoulders, elbows, and finger joints.

Hawkins and Smith[28] found, in a family of 36 members, the family triad of dysplasia of fingernails, arthrodysplasia of elbow joints, and rudimentary patellae in 18 members. Other developmental anomalies were present in fewer members.

Wildervanck[29] described a family of 53 members, 22 of whom showed anomalies in different combinations of the elbows (luxation, subluxation, or malformation of the capitulum radii, insufficient development of the capitulum humeri, exostoses of the ulna), of the knees (luxation, absence or hypoplasia of the patella), and of the nails (absence or dystrophy).

Comment. So far as changes related to the knee are concerned, special significance is attached to absence of the patellae, because this

seems to support strongly the view that the condition is due to faulty development. In keeping with the present hypothesis it is suggested that intrinsic factor acting locally may influence the development of various manifestations of arthrogryposis multiplex as well as of some examples of the associated conditions such as absence of bones (*e.g.*, radius or patella), polydactyly, dysplasia of nails, cerebral malformation, and mental deficiency.

Cerebral Malformation

Josephy[71] said that microgyria, or more correctly micropolygyria, is a rather frequent malformation, commonly occurring in feeble-minded patients. He described, in a microcephalic white female infant, 9 months of age, with spasticity of the extremities, a small agyric brain with corpus callosum completely missing. He pointed out that most brains without a corpus callosum show a well developed gyral pattern so that the defects of the corpus callosum are not necessarily connected with the agyria. He said the cause of the malformation may be either intrinsic or extrinsic.

Sachs,[72] in his original paper on arrested cerebral development, described the brain of a girl who died at the age of 2 years. On the mother's side there was a strong hereditary predisposition to mental disease and several relatives of the father had developed various forms of insanity. At necropsy the most striking features of the outer surface of the left cerebral hemisphere were the depth of all the fissures and the comparative simplicity of the fissuration. The convolutions were alternately narrowed and broadened. Sachs regarded the condition as one of agenesis pure and simple, and he was unwilling to speculate as to its cause although he said the factor of traumatism could not be overlooked.

Sometimes intracranial lipoma is associated with microgyria. The microgyria may be in juxtaposition with the lipoma (Scherer[73]) or at a distance from the lipoma (case 1 of Vonderahe and Niemer[74]).

Greenfield and Wolfsohn[75] described 2 brains illustrating the agyric form and the complex microgyric form. They considered the anomalies to be of the same generic kind. Tredgold[76] published a photograph of a microcephalic imbecile, 33 years of age, who had two thumbs on the right hand. Photographs of the brain showed it to be small and simply convoluted. Garrod *et al.*,[77] in describing the alterations of cerebral diplegia (congenital spastic paralysis—Little's disease), showed photographs of brains in which agyria and microgyria were conspicuous features.

My attention was drawn recently to a case (P. U. S. 7051) of

cerebral spastic quadriplegia with athetosis. The patient, a female child, had polydactylism; the extra digits (halluces and thumbs) were rudimentary and functionless.

Collier[78] said that cases of postnatal diplegia of insidious onset and clinically similar to the prenatal cases were recorded as having their onset even as late as the twelfth year. He also said that families in which prenatal and postnatal cases occurred indiscriminately have been recorded many times. Diplegia, he added, may also, in rare instances, be coincident in the same families with other degenerative diseases of the nervous system, notably hereditary ataxia and myopathy. The cause of the initial process, said Collier, is entirely mysterious as is true of many degenerative nervous diseases.

Comment. While recognizing that agyria and microgyria may in some instances be due to extrinsic factors acting during embryonic life, it is suggested that intrinsic factors may underlie microcephaly and various malformations of the brain including agyria and microgyria; that when microgyria is associated with intracranial lipoma intrinsic factor may predispose to them both; that when abnormalities of limb-buds are associated with malformations of the brain, whether of extreme degree as in exencephaly (*vide* Streeter's[3] case 5342) or of mild degree as in agyria (Tredgold's[76] case *vide supra*), intrinsic factor at the basic level may have predisposed to them both; that in Sachs'[72] case intrinsic factor may have predisposed to the cerebral malformation of the patient as well as to the diseases of the nervous system in the relatives; that in cerebral diplegia, whether stationary or progressive, whether prenatal or postnatal, intrinsic factor may be the predisposing cause in some cases; that intrinsic factor may have influenced the development of the polydactyly and the abnormality of the nervous system presumably present in the case of cerebral spastic quadriplegia with athetosis (P. U. S. 7051); that when other diseases such as myopathy occur in members of a family, one of whose members is diplegic, intrinsic factor may have been a predisposing cause of these other diseases; that in case 8 in the present paper intrinsic factor at the basic level may have influenced the development of the abnormalities in the inferior extremities as well as those in the brain.

Summary and Conclusions

According to the hypothesis advanced in this paper, intrinsic factors are capable, not only of influencing the production of static or stationary lesions (such as may be produced by extrinsic factors), but also, by acting at intervals or continuously before and/or after birth,

of influencing the production of lesions that are dynamic or progressive.

Dynamic or progressive lesions may be characterized by increase or diminution of bulk. It is with those showing diminution of bulk that the present paper is concerned. The conditions chosen for study are (1) agenesis of digits, (2) facial hemiatrophy, (3) cerebral agyria and microgyria.

For certain other diseases which may occur in direct or indirect association with those three pathologic conditions it is suggested that the association (in many instances at least) is not fortuitous. These other diseases include scleroderma, local panatrophy of Gowers, Raynaud's disease, acrocyanosis, hereditary hemorrhagic telangiectasia (Osler-Rendu disease); otosclerosis, deaf-mutism, osteogenesis imperfecta, cutaneous lentigines, melorheostosis, muscle dystrophy, spastic quadriplegia with athetosis, arthrogryposis multiplex congenita, icthyosis palmaris et plantaris, dysplasia of nails, Hirschsprung's disease, hypertrophic stenosis of the pylorus, the phacomatoses, arachnodactyly, dystrophia adiposo-genitalis, and mental disease associated with various malformations of the nervous system.

The essence of the present hypothesis is that many examples of those three pathologic conditions, and of the other diseases that may be directly or indirectly associated with them, are due, not to extrinsic factors, traumatic, infectious, nutritional, or actinic, not to defective hormonal control, not to disturbances of innervation, but to local inferiority of tissue in various parts of the body resulting from influences transmitted in the germplasm and determined by fundamental biologic laws of growth and development.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 74

FIG. 1. Case 1. This photograph is taken from the paper by Dr. George L. Streeter[3] by permission of the Carnegie Institute of Washington. The left index finger and thumb are greatly enlarged, the right forearm and hand are enlarged, and the right thumb and index finger are smaller than normal.

FIG. 2. Case 2. The skiagram shows malformation of the right hand (including absence of some digits and corresponding metacarpal bones.)

FIG. 3. Case 3. Congenital defects of the hands due to agenesis, amputation, or both.

FIG. 4. Case 3. Congenital defects of feet, especially the right. The defects of the left foot were less conspicuous than those of the right, and are scarcely visible from the plantar aspect. There is an ulcer on the sole of each foot.

1

2

3 4

Inglis Agenesis and Deficiency of Parts

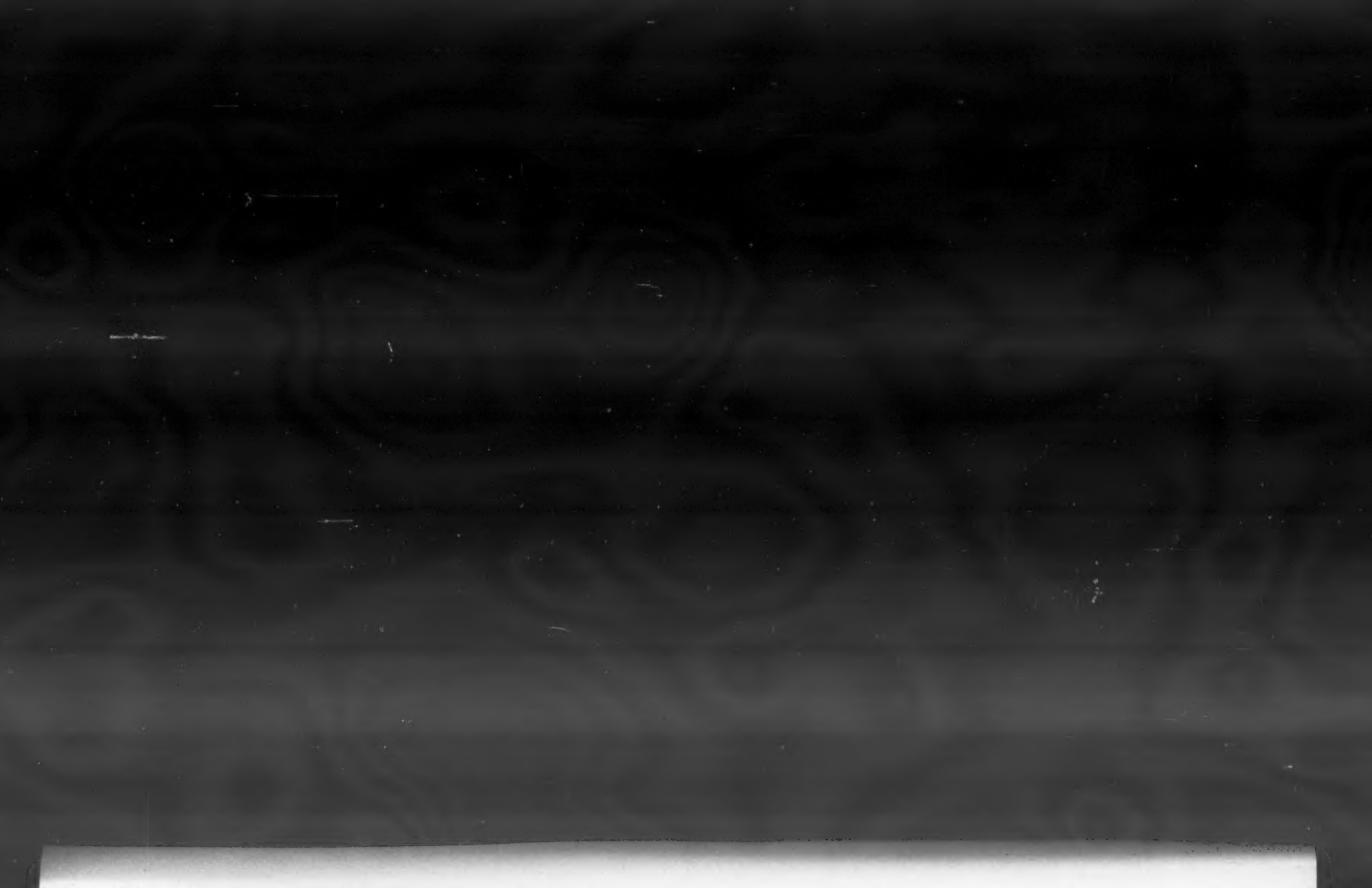
Plate 75

Fig. 5. Case 4. The toes of the left foot, except the great toe, are webbed and considerably smaller than normal.

Fig. 6. Case 5. The right foot and its toes are greatly shortened.

Fig. 7. Case 6. Lentigines and larger pigmented areas are seen on the back, neck, and arms.

Fig. 8. Case 6. Lentigines and larger pigmented areas are seen on thighs, legs, feet, and hands.



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8

Inglis

Agenesis and Deficiency of Parts

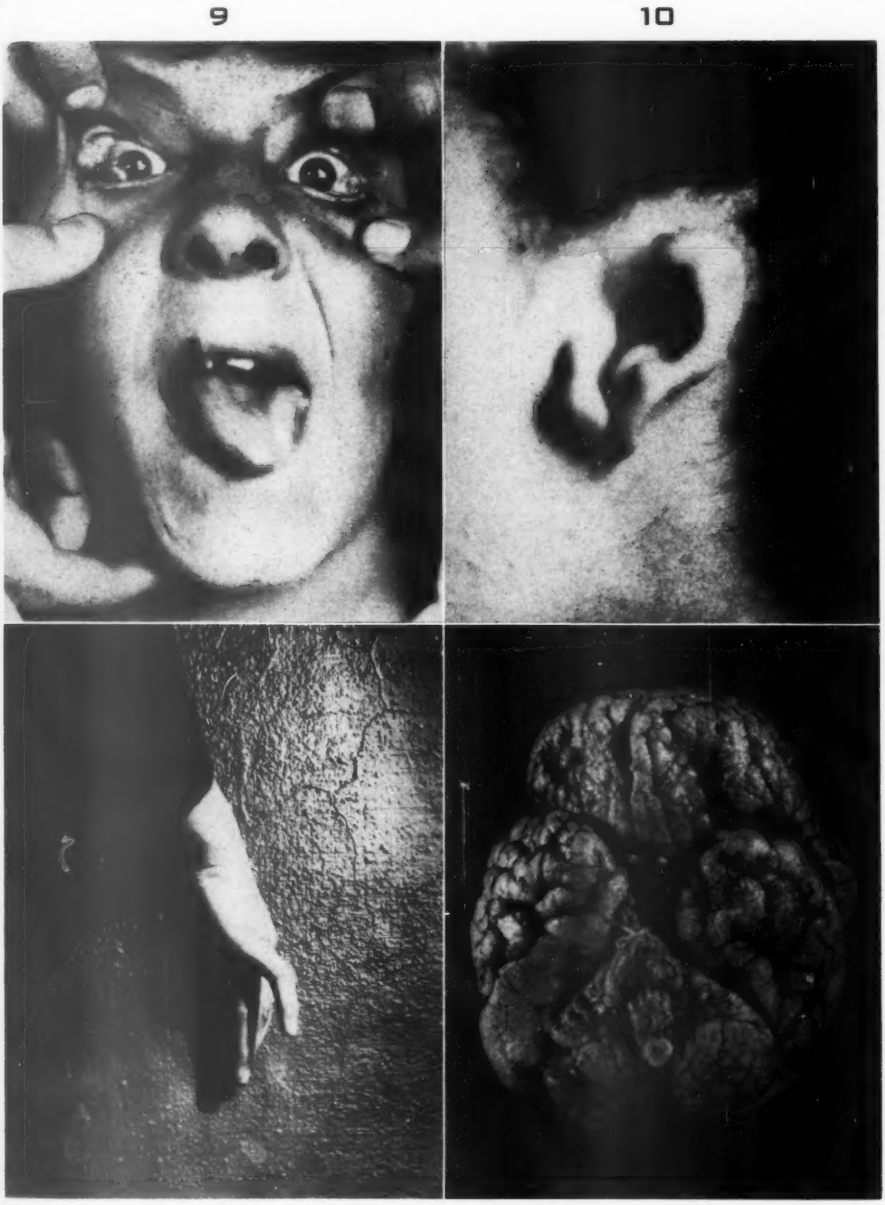
PLATE 76

FIG. 9. Case 7. The left side of the face and the left side of the tongue are smaller than the corresponding parts of the right side. The tip of the tongue is directed to the left. A tumor is present on each eyeball near the outer canthus.

FIG. 10. Case 7. The left ear is malformed; an accessory lobule is present just in front of the tragus, and the helix is unduly curved over.

FIG. 11. Case 7. This shows flexion of the right little finger. Both little fingers were kept in a partly flexed position.

FIG. 12. Case 8. On the inferior surface of the brain there is conspicuous evidence of defective development. In particular, on the right side of the cerebrum posteriorly there is an area showing little evidence of convolutional pattern, and on the right side of the cerebrum anteriorly microgyria is seen.



Inglis Agenesis and Deficiency of Parts

THE COMPARATIVE MORPHOGENESIS OF THE DERMO-EPIDERMAL NEVI AND MALIGNANT MELANOMA *

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There have been numerous studies concerning the relationship between the dermo-epidermal nevi and malignant melanomas. Authors have developed various theories in attempts to explain the histogenesis of pigmented nevi and malignant melanoma. Few of these concepts, however, satisfactorily explain many of the apparent paradoxes that arise in the analysis of this group of tumors. As Lund and Stobbe[1] emphasized, the full significance of the nevi and malignant melanoma can be realized only after adequate correlation with histogenesis, age, and anatomical location.

The origin of the pigmented nevi and malignant melanoma is still controversial. Masson[2] believed that the cellular nevi arise by: (1) proliferation of the intra-epidermic melanoblasts, and (2) a proliferation of the schwannian cells of the dermic nerves. Allen,[3] on the other hand, supported the epidermal origin of the nevi and the malignant melanomas. He explained the presence and over-production of the melanoblasts on the basis of transformation primarily of cells of the malpighian, and, to a lesser extent, of the granular layer.

Until Lund and Stobbe[1] presented their excellent analysis based on "the natural history of the pigmented nevus," there had been a paucity of studies utilizing a large number of cases, or special histologic technics.

The present investigation is based on the belief that additional causative relationships between these tumors and their sites of origin might be uncovered by study of serial sections stained by specific technics. These data are correlated according to the chief criteria of Lund and Stobbe.[1]

Method and Results

All skin lesions examined in the Department of Surgical Pathology from 1945 to 1950 inclusive were reviewed. A total of 450 pigmented lesions were available for study, and approximately 10 per cent were selected for detailed analysis because of the availability of sufficient tissue and adequate clinical data. These lesions were chosen likewise because of their clear-cut adherence to the various nevus types proposed by other authors in order to permit valid comparisons with those authors.

* Received for publication, September 7, 1951.

Numerous varied classifications of the nevi have been presented, but none is entirely satisfactory, since transitional forms exist between the various types.

Lund and Stobbe[1] differentiated their series of 200 nevi into five types:

"A. The nevus consists mostly of intra-epidermal cells and cells at the dermo-epidermal junction . . . . [with] few nevus cells in the corium.

"B. The nevus consists of intra-epidermal cells and cells at the dermo-epidermal junction and there are nevus cells in fair number in the corium.

"C. The nevus consists mostly of nevus cells in the corium but there is still moderate to marked cellular proliferation in the epidermis or at the dermo-epidermal junction.

"D. The nevus consists of nevus cells and there is no, or very little, junctional proliferation.

"E. This corresponds to type D except that many of the nevus cells are fibrillar in type."

Traub and Keil[4] and Allen[3] have subdivided the nevi into: intradermal, junctional (dermo-epidermal), and compound types. In the intradermal nevus, or common mole, the main nevic component is limited to the corium or subepidermal zone. This type usually has a considerable free zone, and there is no dermo-epidermal or junctional change (corresponds to types D and E of Lund and Stobbe[1]). The junctional or dermo-epidermal nevus is characterized by proliferation of cells at the dermo-epidermal margin with few or no cells in the corium or subepidermal zone (type A of Lund and Stobbe). The compound nevi include those lesions which have both a junctional and an intradermal, or subepidermal component (types B and C of Lund and Stobbe).

In order to permit a detailed analysis in a fairly simple form we are utilizing the classification of Traub and Keil[4] and Allen[3] correlated with Lund and Stobbe's[1] viewpoints. In the present study, accordingly, all pigmented lesions were classified as: (1) junctional (dermo-epidermal); (2) intradermal (subepidermal with free zone); (3) compound (combinations of 1 and 2); and (4) malignant melanoma.

Since most authors agree that the intradermal (subepidermal) nevi are rarely the source of malignant melanoma, attention was focused primarily on the junctional and compound type. Blue nevi were excluded also.

The material on which this study is based, therefore, consists of 7 junctional nevi, 30 compound nevi, and 12 malignant melanomas. Of the malignant melanoma group, 7 cases showed metastases to lung or

regional lymph nodes at the time or shortly after removal of the primary skin lesion.

Multiple serial sections were prepared at 5 μ thickness, and a minimum of 15 sections of each lesion was obtained. Sections of each specimen were stained with Masson's trichrome, Heidenhain's iron hematoxylin, Morgan's stain for myelinated nerve fibers (essentially a modification of Heidenhain's iron hematoxylin), Davenport's stain for terminal nerve endings, and ammonium silver nitrate for melanin.

The dermo-epidermal and compound nevi, and the malignant melanomas were subdivided according to the topographic classification of Lund and Stobbe[1] as to: (1) head and neck, excluding ocular and conjunctival lesions; (2) trunk, including external genitalia and perineum; (3) proximal extremities (shoulders, arms, buttocks, and thighs); (4) distal extremities (forearms, hands, legs, and feet).

These four groups were analyzed according to the following criteria:

a. General morphologic characteristics such as shape, pattern, and cell types, presence of mitotic figures, and multinucleation.

b. Junctional proliferation.

c. Melanin distribution.

d. Histioid pattern, including relationship to nerve components, neuroid bundles and nerve-like elements, and skin appendages.

e. Miscellaneous observations, including inflammation, multiple nevi, and nevi associated with pregnancy.

General Morphologic Characteristics

All lesions were divided into flat, or only slightly elevated, and polypoid, or papillary groups. The distribution of these groups and their correlation with age are shown in Table I. All junctional nevi were flat. The majority of the compound nevi were polypoid. The malignant melanomas were predominantly flat, although three slightly elevated forms were found. These three exceptions occurred on the trunk or proximal extremities. No polypoid melanomas were found on the distal extremities. There was no significant statistical difference in the age distribution between the flat and polypoid types.

The correlation between type of lesion and anatomical site is shown in Table II. The junctional nevi were most common on the distal extremities in all age groups. The compound nevi occurred most often on the face and trunk and most commonly in the second and third decades. The distribution of the malignant melanomas was fairly uniform anatomically. Slightly increased incidence was noted on the distal extremities. This occurred in the fourth and fifth decades.

Pattern and Cell Types. The clear cells and other cells of the junc-

tional nevus are by definition largely confined to the dermo-epidermal margin. Occasionally, isolated clumps and groups of such elements were found scattered throughout the superficial corium.

In the compound nevi the more deeply lying cells throughout the corium were arranged in nests and cords, and often in alveolar fashion.

TABLE I

Age Distribution According to Shape

Age (years)	Type						Total
	Junctional		Compound		Malignant melanoma		
	F	P	F	P	F	P	
0–9	2	0	2	1	0	0	5
10–19	0	0	4	3	0	0	7
20–29	1	0	4	5	1	0	11
30–39	3	0	2	7	0	0	12
40–49	1	0	1	1	2	1	6
50–59	0	0	0	0	2	2	4
60–	0	0	0	0	4	0	4
Totals	7	0	13	17	9	3	49

F= flat or slightly elevated.
P= polypoid or papillary.

The individual cells were generally smaller than those of the dermo-epidermal junction. Their nuclei were relatively large with a delicate vesicular chromatin pattern, and occasional nucleoli. The cytoplasm was abundant, sharply defined, and clear or slightly eosinophilic. In a few instances transitional forms between the clear cells of the dermo-epidermal margin and the more deeply lying smaller cells were apparent.

Among the malignant melanomas there was considerable variation in the cellular pattern. Two lesions exhibited atypical hyperplasia of

TABLE II

Distribution According to Anatomical Site

Anatomical site	Type			Total
	Junctional	Compound	Malignant melanoma	
Head and neck	0	8	3	11
Trunk	1	15	3	19
Proximal extremity	2	5	2	9
Distal extremity	4	2	4	10
Totals	7	30	12	49

cells along the junctional zone associated with the formation of well defined epithelial pearls. These lesions, which simulated squamous carcinoma, will be discussed in detail later. Three of the melanoma group demonstrated an alveolar pattern. Five lesions showed broad sheets of eosinophilic polygonal cells diffusely distributed throughout the corium and deeper layers. Two lesions showed a pattern resembling a neurofibroma, with intermixture of spindle cells and nerve elements. In general, it was found that the pattern of the malignant melanoma may simulate squamous cell carcinoma, adenocarcinoma, or neurofibroma. The individual cell types exhibited considerable pleomorphism. The most common cell type was a large, plump cell vaguely resembling liver cells. Nuclei were large, and the nuclear chromatin usually clumped. Vacuolization was common. Nucleoli were large and prominent. The cytoplasm was abundant, and the cell boundaries usually sharply defined. Spindle-shaped cells also were common, and in most instances the cytoplasm of these cells was poorly defined and occasionally fibrillar.

Mitotic Figures. The number of mitotic figures in each lesion was graded quantitatively as follows: few, 0 to 3 mitotic figures per high-power field; moderate, 3 to 10 per high-power field; numerous, 10 or more per high-power field.

The junctional and compound nevi contained few mitotic figures. Occasional mitotic figures were noted in the cells along the marginal zones, but none was of the atypical or bizarre form.

In the malignant melanoma group, 4 lesions showed few mitotic figures, 6 showed moderate numbers, and 2 showed numerous mitotic figures. Most of the mitotic figures were bizarre and atypical.

Multinucleated and Giant Cells. As Lund and Stobbe[1] pointed out, multinucleated cells occurring in the nevi are generally of two types. One is characterized by small aggregations of peripherally located nuclei. The nuclei closely resemble the nuclei of the surrounding nevus cells. The cytoplasm may be poorly defined. The second form of multinucleated cell is larger. Its cytoplasm is sharply defined, and the nuclei may be either central or peripheral in arrangement.

In the junctional nevi multinucleated and giant forms were extremely scarce. In the papillary compound nevi of the face and trunk, throughout the third and fourth decades, they were especially prominent. They were few or absent in nevi of the distal extremities.

Eight of the malignant melanomas contained multinucleated cells in moderate numbers and giant cells to a variable degree. The nuclei varied from two to six, and resembled those of the surrounding tumor

cells. Four lesions showed only few multinucleated cells and practically no giant forms.

Spitz[5] stated that the juvenile melanoma may be distinguished histologically from the adult malignant melanoma in one-half the cases by the presence of giant cells in the former, while they seldom occur in the latter. This point is debatable, since multinucleated and giant cells were easily identified in 8 of the 12 malignant melanomas in the present study.

Junctional Proliferation

According to Allen,[3] the junctional or dermo-epidermal nevus is characterized by cells within the epidermis which show evidence of having lost varying degrees of cohesion with adjacent cells. This change is most pronounced in the basal layer, and the involved cells subsequently drop off into the underlying corium (Abtropfung).

There is little or no agreement among the various authors as to the site of origin and the true identity of the junctional cells. Becker[6] concluded that the specialized cells at the dermo-epidermal margin are actually melanoblasts. Masson[2] was in agreement with this concept, and maintained that there is an over-production of melanoblasts. The basic problem centers about the site of origin of the melanoblast. Masson ascribed it to the neural crest, or epiblast. Allen[3] believed that they arise by transformation of the malpighian cells, or from cells of the granular layer of the epidermis.

In the present study there were slight minor variations in the type of proliferation in the junctional and compound nevi. The earliest junctional nevus, removed from the thigh of a 1-week-old infant, showed several foci of junctional proliferation extending over a distance of about 0.4 cm. In the sections stained with ammonium silver nitrate, there were increased numbers of cells resembling melanoblasts in these foci. Most of these cells had poorly defined dendritic processes and vague cell boundaries. Often these cells were closely intermingled with the basal cells, and in some sections the heavy deposition of melanin in the basal cells tended to obscure the dendritic cells. A similar lesion removed from the foot of a 3-year-old child showed the same type of proliferation associated with increased numbers of dendritic cells. In the junctional nevi of the older age groups, the pigmentation of the basal cells was intense and virtually obscured the dendritic cells.

The changes observed in the compound nevi roughly paralleled those of the junctional nevi. Generally, the dendritic cells were most numer-

ous in the junctional foci in the younger age groups, and tended to be obscured and poorly defined in the older nevi.

The foci of junctional proliferation in both the junctional and compound nevi were multicentric in origin in almost every lesion. In the junctional nevi, these foci involved relatively short stretches of the epidermis and averaged 2 to 5 mm. in diameter. In the compound nevi, the foci of proliferation involved longer stretches, averaging 4 to 8 mm. The papillary compound nevi of the third and fourth decades frequently demonstrated well defined foci of multicentric proliferation in the superficial papillary tips.

In the malignant melanoma group, junctional proliferation was marked in 9 of the 12 lesions. In most instances the junctional changes involved extensive stretches of the epidermis, rather than occurring in multiple small foci as in the junctional and compound nevi.

Allen[3] placed great importance on the junctional change in malignant melanoma, and stated that the absence of such a change may be used reliably to differentiate a primary from a metastatic melanoma of the skin. Lund,[7] however, pointed out that the absence of junctional changes does not necessarily preclude the diagnosis of primary malignant melanoma. In lesions that are severely ulcerated, or have been previously traumatized by surgery, electrocautery, or caustic chemicals, the junctional component may be obscured or absent.

In the present study 4 of the malignant melanomas showed marked acute ulceration, and in 3 of these lesions, the junctional changes were slight or absent. In these cases, however, the histologic characteristics were sufficient to warrant a diagnosis of primary malignant melanoma, confirmed by the subsequent development of metastases

Clear Cells

The cells of the dermo-epidermal margin are often referred to as the clear cells. These cells are especially prominent in the foci of junctional proliferation, but may be found within the basal layer along hair follicles and sweat glands. Masson[2] believed that these cells correspond to the perikaryon of the melanoblasts and are normally found in the epidermis. He also stressed that these cells may be confused with other similar cells, such as granulocytes, monocytes, or cancer cells.

In this study both the nevi and the malignant melanomas demonstrated numerous clear cells throughout the junctional foci. In most instances there was no clear-cut distinction between the nevic and melanoma cells, and transitional forms were numerous. With the

ammonium silver nitrate stain, these cells were found to contain sparse amounts of melanin pigment dispersed throughout the cytoplasm. It cannot be proved definitely, but the presence of melanin within these cells and their arrangement lend support to the concept that these cells are intimately related to the melanoblasts.

Two of the malignant melanomas showed unusual deviations from others in the group with respect to junctional changes. One occurred on the toe of a woman, 43 years old, who died of generalized metastases 4 months following removal of the primary lesion. The other occurred on the heel of a man, 58 years of age, who developed inguinal metastases 6 months following surgery. Both of these lesions demonstrated numerous foci of junctional proliferation with apparent transitions from basal cells to tumor cells. Closely associated with the junctional component, however, was a marked proliferation of the prickle cell layer with the formation of well defined epithelial pearls. Transitional forms between the epithelial nests and the neoplastic cells also were numerous. In both lesions the atypical epithelial hyperplasia may well represent an abnormal stimulation produced by the malignant melanoma.

Melanin Distribution

In both the junctional and compound nevi, melanin was present predominately throughout the zones of junctional proliferation, and to a lesser extent throughout the subjunctional zone. Only rarely was melanin distributed throughout the nevic cells in the deeper portion of the corium. In no instance was melanin found in the nevic cells closely associated with hair follicles, sweat, or sebaceous glands.

The malignant melanomas showed heavy depositions throughout the junctional and subjunctional zones. Three lesions demonstrated abundant melanin pigment within tumor cells deep in the corium. Two showed patchy distribution, and two showed only slight melanin deposits throughout the main tumor.

Nerve Components and Perineural Arrangement

Soldan[8] was one of the first to demonstrate that myelinated nerves penetrate nevic tissue, and that the terminal unmyelinated fibers scatter and become unidentifiable. Laidlaw and Murray[9] as well as Foot[10] have shown that nevi are rich in nerve fibers.

In an attempt to follow the course of the smaller unmyelinated nerve fibers, Davenport's stain was used, which proved unsatisfactory. Masson[2] stated that neurofibrillar technics are unpredictable, and par-

ticularly in the nevi. Heidenhain's iron hematoxylin and Morgan's stain gave the most consistent demonstration of the dermic and nevic nerves. The following observations are based largely on results obtained with these stains in combination with Masson's trichrome stain.

The junctional nevi of the first decade generally showed prominent superficial myelinated fibers, which extended almost to the dermoepidermal margin before branching into fine, untraceable fibers. The myelinated nerve fibers appeared normal in number. The earliest junctional nevus (removed from the thigh of a 1-week-old infant) demonstrated several myelinated nerve bundles extending almost to the junctional zone. In this case, numerous clumps of cells, which resembled the cells of the junctional zone, were arranged about the terminal portion of the superficial nerve elements. A few of these cells were deeply pigmented. The most striking finding was proliferation of cells within the perineural sheath. These cells had a relatively large vesicular nucleus, poorly defined cytoplasm, and resembled the syncytial cells of the nerve sheath (Schwann cells). In several sections the cells within the nerve sheath appeared to represent transitional forms between the nevic cells along the marginal zone and the syncytial cells. Similar perineural arrangement and transitional forms were noted in other junctional nevi observed in the first decade.

The compound nevi of the first decade showed similar changes, with prominent myelinated fibers extending throughout the subjunctional zones, perineural arrangements, and proliferation of nevic cells. The compound nevi of the third and fourth decades showed several differences in the distribution of the nerve elements. The superficial nerves were few. The myelinated bundles throughout the corium, however, were prominent, and definitely increased in number. Frequently three or four large myelinated bundles were arranged in plexiform fashion. This change was associated with marked perineural proliferation of the nevic cells. The perineural arrangement and proliferation were especially marked in the papillary compound nevi of the third and fourth decades.

It might be argued that the perineural proliferation represented trapping of the dermic nerves during the growth and development of the nevus, and that it is not a significant morphogenetic finding. By the examination of serial sections, however, the perineural arrangement and proliferation were often noted in regions remote from the main portion of the nevus. Frequently, the foci of perineural nevic cell proliferation were found at varying depths within the corium and

often lateral to the main nevus. This would invalidate the theory of cellular trapping of the dermic nerves during the growth of the nevus, and suggests the primary perineural origin of the cells.

In the malignant melanoma group, superficial nerve fibers were few or absent, and these were usually obscured by the neoplastic cells. Myelinated nerve bundles were demonstrated deep within the tumors, and more than one-half of the malignant melanomas showed some degree of perineural arrangement of the cells. In the estimation of such

TABLE III

Degree of Perineural Proliferation According to Type of Lesion and Anatomical Site

	Type			Total
	Junctional	Compound	Malignant melanoma	
Head and neck				
None-slight	0	3	0	3
Moderate-marked	0	5	3	8
Trunk				
None-slight	1	9	3	13
Moderate-marked	0	6	0	6
Proximal extremities				
None-slight	0	2	0	2
Moderate-marked	2	3	2	7
Distal extremities				
None-slight	1	2	1	4
Moderate-marked	3	0	3	6
Totals				
None-slight	2	16	4	22
Moderate-marked	5	14	8	27
	7	30	12	49

changes in this group, care must be taken not to mistake perineural lymphatic metastases for perineural arrangement.

Two specimens of the malignant melanoma group showed a more mature type of neural differentiation than other members of the group. One specimen was removed from the leg of a man, 57 years old; the other from a supraorbital ridge of a woman, 62 years of age. These lesions demonstrated arrangement of cells in interlacing bundles with closely intermixed nerve fibers. Histologically they resembled neurofibroma or neurofibrosarcoma. Each showed little or no junctional change of the overlying epithelium, but both demonstrated metastases.

Table III is a summary of the degree of perineural proliferation and arrangement of the various groups according to anatomical site.

Neuroid Bundles and Nerve-like Elements

Frequently the nevi demonstrate, deep within the corium, abundant fibrillary material resembling neurofibrils and closely associated with the nevic cells. Masson[2] referred to these elements as neuroid bundles, because of their histogenetic resemblance to the schwannian syncytium. Other peculiar structures may be found which resemble the tactile corpuscles of Wagner-Meissner. Masson[2] divided these into two forms: one a simple form, the foliated lamina; the other complex, the nevic corpuscle. According to Masson, the foliated lamina are invisible in hematoxylin and eosin stains. He stated that the trichromes and silver impregnation of the cytoplasm are necessary for their demonstration. In the present study, structures resembling the foliated lamina were demonstrated not only by the trichromes, but also by Heidenhain's iron hematoxylin.

TABLE IV

Frequency of Neuroid Bundles and Nerve-like Elements According to Type of Lesion and Anatomical Site

	Type			Total
	Junctional	Compound	Malignant melanoma	
Head and neck				
None-few	0	5	2	7
Moderate-many	0	3	1	4
Trunk				
None-few	1	8	3	12
Moderate-many	0	7	0	7
Proximal extremity				
None-few	2	2	2	6
Moderate-many	0	3	0	3
Distal extremity				
None-few	4	2	2	8
Moderate-many	0	0	2	2
Totals				
None-few	7	17	9	33
Moderate-many	0	13	3	16
	7	30	12	49

Table IV is a tabulation of the frequency of neuroid bundles and nerve-like elements throughout the different groups, arranged according to anatomical site. The foliated lamina are included also. Neuroid bundles and nerve-like elements were especially common in the papil-

lary compound nevi of the third and fourth decades, and scarce in the flat, junctional nevi of all ages.

As Masson[2] emphasized, the foliated lamina and the nevic corpuscles may develop in any part of the skin except the finger and toes (the sites normally occupied by the Wagner-Meissner corpuscles). This observation was substantiated, as these structures were not found in nevi of the distal extremities.

In the malignant melanoma series, neuroid bundles and nerve-like

TABLE V

Degree of Perifollicular Proliferation According to Anatomical Site

	Type			Total
	Junctional	Compound	Malignant melanoma	
Head and neck				
None-slight	0	2	3	5
Moderate-marked	0	6	0	6
Trunk				
None-slight	1	10	3	14
Moderate-marked	0	5	0	5
Proximal extremity				
None-slight	2	4	2	8
Moderate-marked	0	1	0	1
Distal extremity				
None-slight	4	2	4	10
Moderate-marked	0	0	0	0
Totals				
None-slight	7	18	12	37
Moderate-marked	0	12	0	12
	7	30	12	49

elements were absent, except for the two lesions which demonstrated a neurofibromatous pattern.

Relationship of Nevi to Skin Appendages

Lund and Stobbe[1] found perifollicular arrangement of the nevic cells especially prominent in children, least conspicuous in the head and neck regions, and more prominent on the proximal and distal extremities.

Several discrepancies were noted in the analysis of the relationship of the nevic cells to skin appendages. The routine hematoxylin and eosin sections often were misleading. The compound nevi frequently included apparent foci of junctional proliferation about hair follicles. Examination of the same lesion with the trichrome stain revealed,

however, that most of the nevic cells were separated from the adjacent follicle by a thin band of collagen. Small foci of junctional proliferation were noted in a few instances along the course of hair follicles, but in none of the involved cells was melanin demonstrated.

Table V shows the degree of perifollicular proliferation correlated with type of lesion and anatomical site. In contrast to other authors, we found that marked perifollicular proliferation was infrequent, and was confined largely to the compound nevi.

Inflammation

The degree of inflammation of the different types was graded as slight, moderate, or marked. Two junctional nevi, occurring on the distal extremities, showed slight chronic inflammation. One compound nevus showed marked acute and chronic inflammation; 6, moderate inflammation; and 8, slight chronic inflammation. None showed ulceration. The nevi occurring on the trunk and extremities, particularly those of the third and fourth decades, showed perifollicular infiltrations of lymphocytes and histiocytes. No significant difference in the degree of inflammation was noted between the flat and the papillary nevi.

Nine malignant melanomas showed marked chronic inflammation, which was diffuse and involved the marginal and superficial portions of the tumor. Ulceration and acute inflammation were present in 4.

Multiple Nevi

Table VI shows the distribution of multiple junctional and compound nevi correlated with age, and compared with the intradermal

TABLE VI

Distribution of Multiple Lesions According to Age

Age (years)	Type				Total
	Intradermal	Compound	Junctional	Malignant melanoma and nevus	
0–9	0	1	0	0	1
10–19	0	1	0	0	1
20–29	3	3	0	0	6
30–39	4	3	0	0	7
40–49	4	0	0	1	5
50–59	0	0	0	0	0
60–	0	0	0	0	0
Total	11	8	0	1	20

nevi. When multiple nevi were removed from the same individual, the type and structure were identical in all lesions. In one case, that of a man, 49 years old, a malignant melanoma was removed from the right leg. A compound nevus also was removed from the same leg proximal to the malignant melanoma. This nevus showed no essential difference from other compound nevi. Most of the cases with multiple lesions showed a very high incidence of the intradermal type. This is to be expected, since statistically the intradermal nevi are most numerous.

Nevi Associated with Pregnancy

Pack and Scharnagel[11] stressed the poor prognosis of pregnant women with malignant melanoma. They concluded, after a study of 32 cases, that the pigmented nevic and melanoma cells are extraordinarily sensitive to hormonal stimulation, presumably of pituitary, adrenal, and ovarian origin.

In the present study 2 compound nevi and one intradermal nevus were removed from different women at the time of delivery. None of these lesions showed histologic evidence of recent growth, or of malignancy. One small malignant melanoma was removed from the face of a woman, 22 years old, who delivered a stillborn child at 6½ months. This lesion did not differ significantly from the other malignant melanomas.

Summary

It is recognized that the separation of the nevi into junctional (dermo-epidermal), compound, and pure intradermal (subepidermal) types is arbitrary, since each may well represent the same process in a different phase of development. By such segregation, however, differences are apparent when the dermo-epidermal, and compound nevi, and malignant melanomas are compared and correlated with these criteria.

In the earliest junctional and compound nevi, the nevic cells tend to differentiate from cells along the dermo-epidermal margin (melanoblasts), and also from cells within the perineural sheath. This differentiation probably continues throughout life, as evidenced by the increase in neuroid bundles, nerve-like elements, and suggestive perineural arrangement of nevic cells in the older nevi.

The malignant melanomas show certain similarities in morphogenesis to the dermo-epidermal nevi, especially in regard to type of junctional proliferation and perineural arrangement of cells.

Masson's[2] concept of the dual origin of the nevi from intraepidermic melanoblasts, and proliferation of schwannian cells is supported by our data.

It is suggested, but not proved, that the variability of pattern exhibited by the malignant melanomas may be related in some manner to a similar dual origin.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

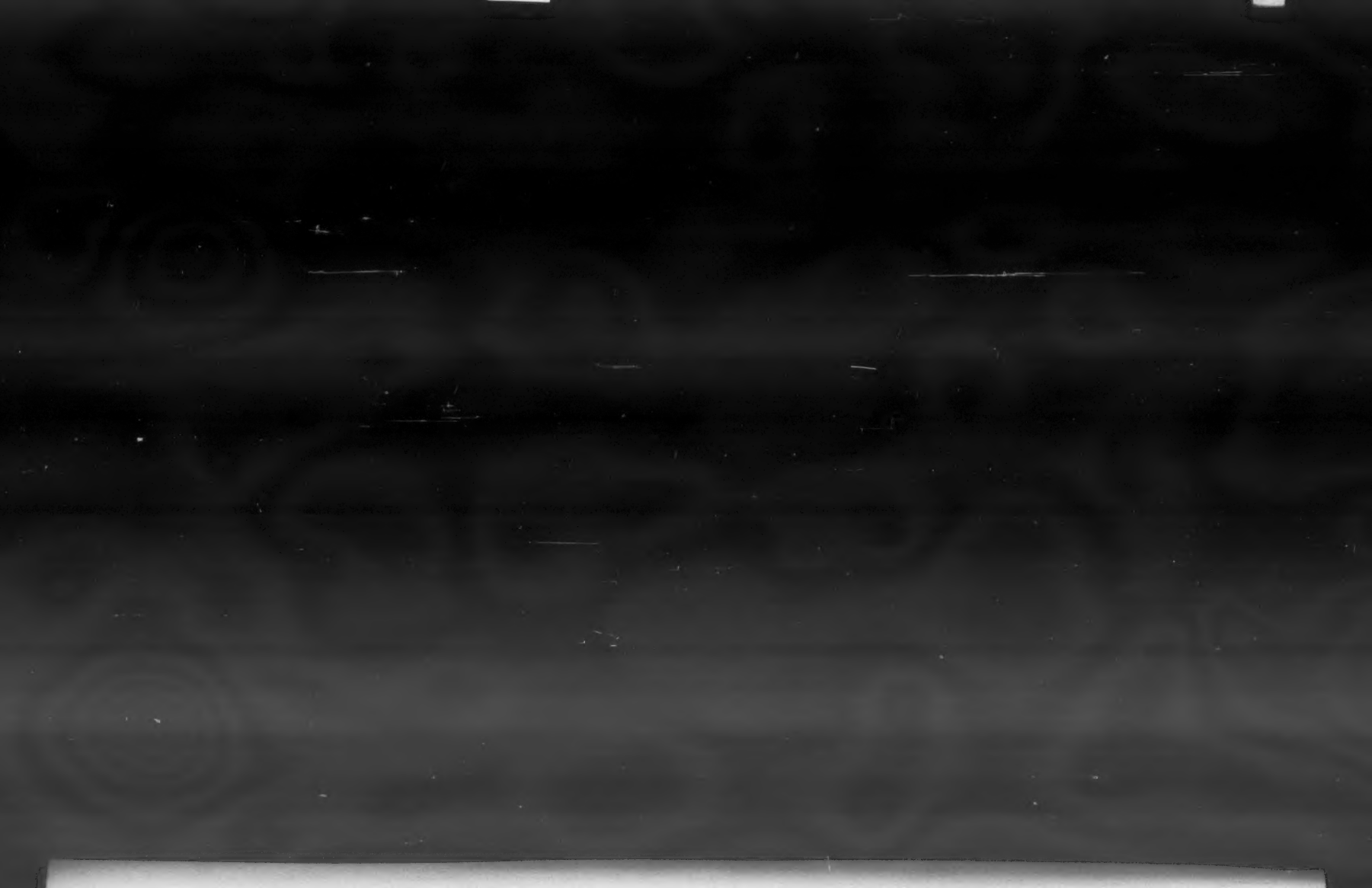
PLATE 77

FIG. 1. Junctional nevus of the mons pubis of a female, 34 years of age. There is heavy deposition of melanin within the cells of the dermo-epidermal margin. Ammonium silver nitrate stain. × 165.

FIG. 2. Same lesion as shown in Figure 1. Several dendritic cells are closely intermingled with heavily pigmented basal cells. Ammonium silver nitrate stain. × 825.

FIG. 3. Junctional nevus of the thigh of an infant, 1 week old. Within the perineural sheath there is proliferation of cells which resemble Schwann cells. Heidenhain's iron hematoxylin stain. × 515.

FIG. 4. Compound nevus of the abdomen of a female, 43 years of age, showing myelinated nerves and perineural proliferation of nevic cells. Heidenhain's iron hematoxylin stain. × 440.



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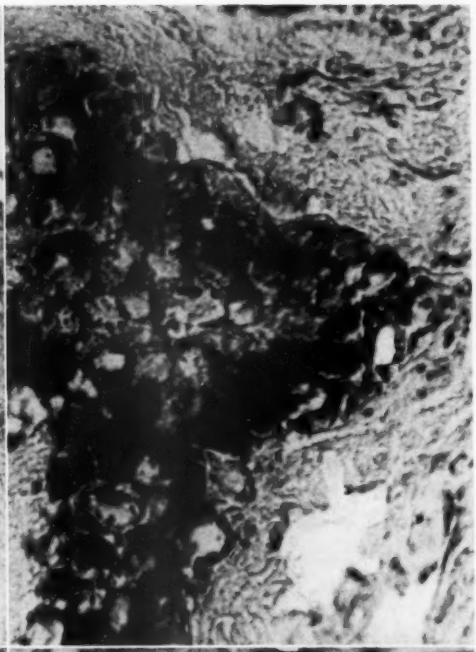

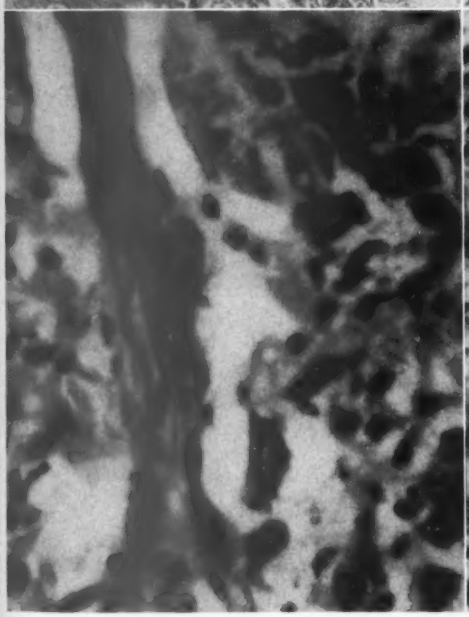

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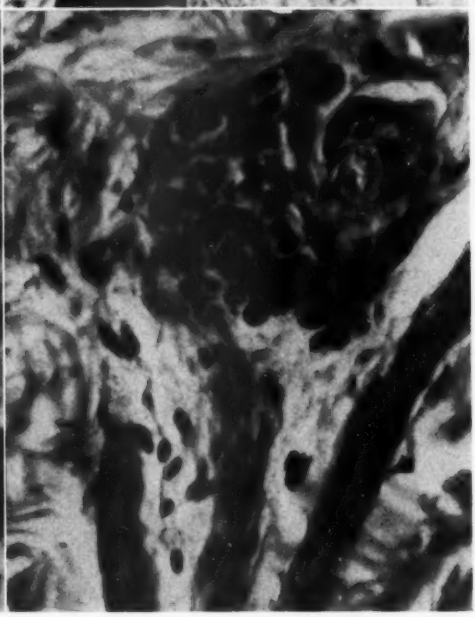
Berkheiser and Rappoport



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Nevi and Malignant Melanoma

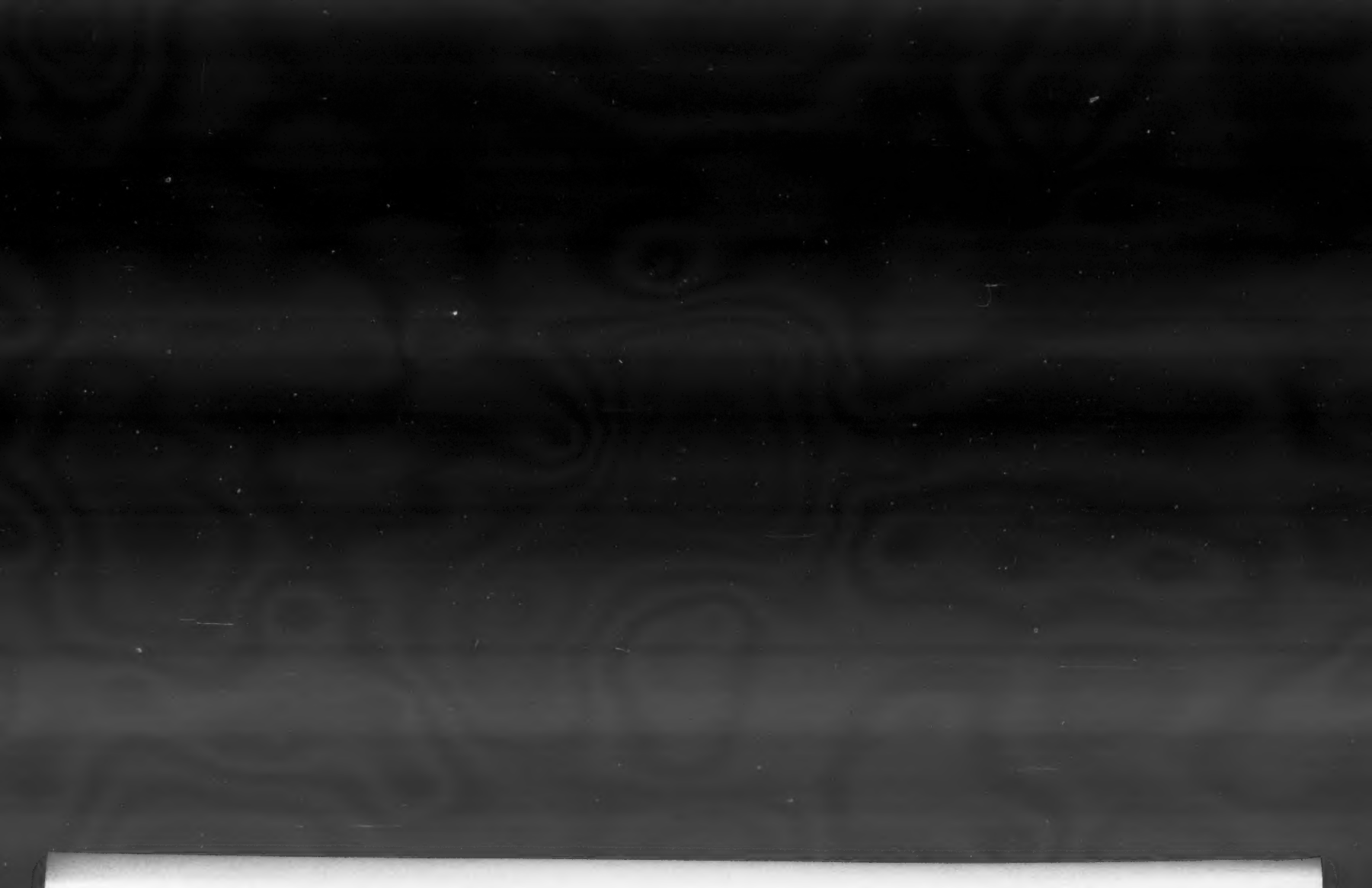
PLATE 78

FIG. 5. Malignant melanoma of the cheek of a pregnant woman, 22 years of age. The junctional change is diffuse, and clear cells are numerous. Heidenhain's iron hematoxylin stain. × 180.

FIG. 6. Same lesion as shown in Figure 5. Near the lower margin there is a myelinated nerve with perineural arrangement of the neoplastic cells. Heidenhain's iron hematoxylin stain. × 180.

FIG. 7. Malignant melanoma of the supraclavicular region of a woman, 60 years of age. There is an intermixture of spindle-shaped cells and nerve elements resembling neurofibroma. Heidenhain's iron hematoxylin stain. × 130.

FIG. 8. Malignant melanoma of the toe of a woman, 43 years of age. There is marked atypical epithelial hyperplasia simulating squamous cell carcinoma. Heidenhain's iron hematoxylin stain. × 65.



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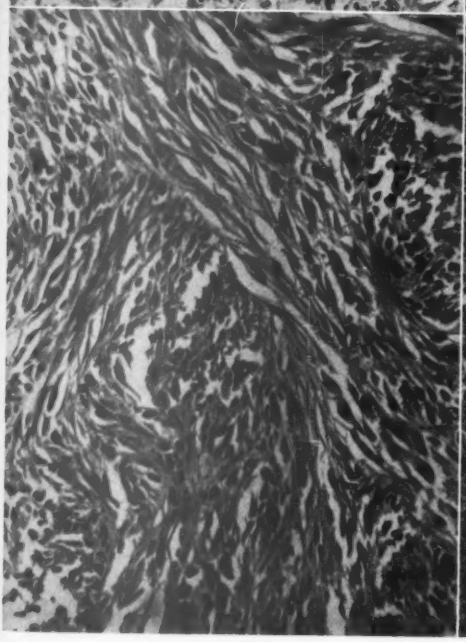

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Nevi and Malignant Melanoma

OBSERVATIONS ON THE PERSISTENCE OF HEPATIC GLYCOGEN AFTER DEATH *

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Although it is well recognized that glycogen disappears from the liver after death, there is still difference of opinion as to how rapidly and completely this change occurs. Because of this, one is unable to evaluate determinations of glycogen in livers received from necropsies. Moreover, the histologic demonstration of glycogen is not readily correlated with amounts determined chemically. The problem is further complicated by conflicting views on the fixation and staining methods to be used for demonstrating glycogen histologically.

This study was undertaken with a view toward determining how rapidly glycogen disappears from the liver after death, as determined by both chemical and histologic methods. We have also attempted to evaluate the various methods of fixation and staining in order to establish their reliability and relative merit.

MATERIAL AND METHODS

Liver samples were obtained from routine necropsies and surgical material from humans and from freshly sacrificed normal adult male albino rats. Pieces of liver were allowed to stand in a moistened airtight glass container at room temperature for periods varying from 10 minutes to 48 hours. Representative blocks were fixed in Zenker's fluid without the addition of acetic acid, 10 per cent aqueous formalin, Carnoy's fluid, and alcoholic formalin (1 part 40 per cent formaldehyde, 9 parts 95 per cent ethyl alcohol). Microscopic sections were stained with Best's carmine method, Lillie's modification of the Bauer technic,[1] and silver stain utilizing lithium carbonate.[2] In most instances, controls were prepared by treating duplicate sections with saliva before staining. Chemical determinations for glycogen were performed according to the method of Good, Kramer, and Somogyi.[3]

RESULTS

In livers from 100 necropsies, 7 surgical specimens, and 16 rats, a total of 204 sections stained by the Lillie-Bauer method[1] were compared with a similar number stained by Best's carmine, and 37 with

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the silver method. All specimens were embedded in paraffin, and no collodionization was done. The Lillie-Bauer and Best's carmine stains gave equally satisfactory results and corresponded closely in the amount of stainable material (presumably glycogen) demonstrated. Excellent stains for cytoplasmic and nuclear glycogen were seen after fixation with alcoholic formalin, although satisfactory and almost equally brilliant stains were obtained after fixation with Zenker's fluid, Carnoy's fluid, and 10 per cent aqueous formalin.

Surgical Biopsy of Human Liver

The surgical specimens of human liver were wedge-shaped pieces removed at operation for diagnostic purposes. Intravenous glucose was administered during the operation to 2 of the 7 patients. Six patients had no food by mouth for 12 hours prior to operation. All livers showed large amounts of glycogen in the cytoplasm of hepatic cells as demonstrated by Best's carmine stain and the Lillie-Bauer stain.[1] In 2, glycogen was seen in a few nuclei situated especially at the periphery of the hepatic lobules. After a fragment of liver was taken for immediate fixation, the remaining block of hepatic tissue was allowed to stand at room temperature in a moist, closed, glass container. At selected intervals, pieces of liver were taken for fixation and sectioning. Three cases were of secondary carcinoma of the liver, 2 of obstructive biliary cirrhosis, one showed subacute hepatitis, and one cirrhosis with primary carcinoma of the liver of hepatic cell type. It was found that specimens allowed to stand aerobically for 6 hours at room temperature before fixation showed little or no decrease in histologically demonstrable glycogen. Moreover, large amounts of glycogen were still present after 24 hours (Fig. 5), although an appreciable decrease had occurred as compared with the immediate or 6-hour sample. Surprisingly enough, appreciable amounts of glycogen were seen also in 2 livers which had remained at room temperature for 48 hours before fixation. In following the disappearance of glycogen in microscopic slides of successive samples of the same block of hepatic tissue, it was noted that the disappearance progresses in a patchy manner. With no apparent relation to topography as far as the liver lobule or site within the block is concerned, glycogen is removed from some groups of hepatic cells completely, whereas in other areas no appreciable decrease is apparent. Glycogen-containing nuclei seen in freshly fixed specimens were absent in the 24-hour specimen.

Sections of the primary hepatic cell carcinoma showed large amounts of cytoplasmic glycogen in tumor cells, with no difference in amount in blocks of tumor which were fixed in alcoholic formalin after stand-

ing 3, 16, or 24 hours at room temperature (Fig. 6). Cytoplasmic glycogen seemed to disappear at a slower rate from the tumor cells than from the non-malignant hepatic cells.

Liver from Human Necropsies

Best's carmine and Lillie-Bauer[1] stains were done on sections of liver from 100 necropsies. The tissues were fixed in Zenker's stock solution without addition of acetic acid. In 6 of a group of 28 livers in which the necropsy was performed 12 or more hours after death, large amounts of glycogen were demonstrable histologically.

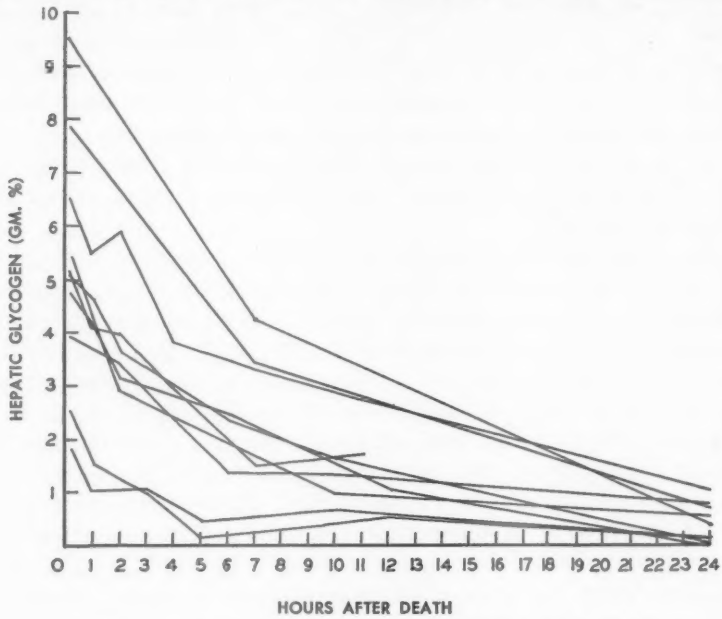
In 12 of 100 livers, no glycogen was demonstrable histologically, in 37 only a trace was present, and in the remaining 51 cases appreciable amounts were seen.

Ten additional livers from human necropsies were fixed in alcoholic formalin, and quantitative chemical determinations of glycogen were performed on unfixed samples of the same livers. In 2 instances, on necropsies performed 19 and 48 hours after death, the livers showed over 1 per cent glycogen. In a third specimen, one of chronic alcoholism with extreme fatty infiltration and portal cirrhosis, satisfactory chemical determinations were not possible because of interference by excess lipids present, and, possibly in addition, paraldehyde, present in amounts sufficient to give a strong odor. In the other 7 cases, glycogen was present in amounts varying from 0.1 to 1.0 per cent in necropsies performed from 14 to 29 hours after death. In the total necropsy series, the rate of disappearance could not be correlated with any particular hepatic disease or condition.

Experimental Animals

Sixteen adult male albino rats, fed up to the time of death, were sacrificed by a blow at the base of the skull. Their livers were removed, and samples were taken immediately for histologic sections and chemical analysis. The remaining liver was allowed to remain at room temperature in a moistened, closed, glass container. At selected intervals up to 24 hours, samples of liver were removed for microscopic and chemical study (Figs. 1, 2, 3, and 4). In 6 rats no chemical determinations were made. The results of the chemical determinations of glycogen are shown in Text-figure 1. Apparently independent of the initial concentration, the most rapid decrease in glycogen, as determined chemically, occurs during the first 6 hours, and proceeds at a slower rate thereafter. This is shown in Figures 1, 2, and 3, in which a reduction in amount of glycogen is seen. Figures 2 and 3 represent fixation after 4 and 24 hours, respectively, as com-

pared to Figure 1, which was fixed immediately. However, there is no appreciable difference in amount between the 4 and 24-hour samples. As previously noted by Grafflin, Marble, and Smith,[4] the amount of glycogen revealed by histologic examination could not be correlated quantitatively with the amount determined by chemical means. This



Text-figure 1. The disappearance of hepatic glycogen in rats after death.

finding was especially striking during the first 12-hour period, when the chemical data frequently showed a marked decrease up to 50 per cent, with little or no change in the amount of glycogen seen microscopically. For example, Best's carmine and Lillie-Bauer[1] stains on livers containing 5.4 and 2.9 per cent glycogen showed apparently equal amounts of glycogen. Moreover, a fall in the chemical value to 1.0 per cent glycogen was reflected histologically as only a slight patchy decrease. The correlation between chemical and histologic estimations of glycogen was equally poor with glycogen levels of less than 1 per cent.

Discussion

Lillie[1] stated that he has observed good glycogen preservation in material refrigerated for 12 to 24 hours before fixation. He also stated that he finds glycogen often beautifully preserved in animal livers fixed after death in buffered neutral formalin. Chipps and Duff[5]

stated that the interval between death and the time of fixation of the liver up to 8 to 10 hours does not materially affect the appearance and quantity of glycogen in the liver, as estimated in sections stained with Best's carmine. This is quite puzzling in view of the fact that according to chemical analysis, glycogen disappears rapidly from hepatic tissue. Vallance-Owen[6] found that storage of rabbit liver at cold temperatures resulted in no appreciable loss of histologically demontrable glycogen for periods up to 48 hours. He also observed that satisfactory glycogen stains may be done on formalin-fixed tissue.

Summary and Conclusions

Histologic methods for the demonstration of glycogen in liver are equally satisfactory after fixation in absolute alcohol, Carnoy's fluid, alcoholic formalin, 10 per cent aqueous formalin, and Zenker's solution without acetic acid.

Best's carmine and Lillie-Bauer[1] stains for glycogen give about equally satisfactory results with all five fixatives. Silver stain with lithium carbonate was found to be unreliable, and gave poor preparations.

Microscopic sections prepared routinely from livers fixed in Zenker's solution or formalin showed little or no decrease in stainable glycogen, when fixation is delayed for as long as 10 hours after death or surgical biopsy.

In necropsies performed as long as 10 hours after death, therefore, the microscopic appearance of glycogen in the liver is a good index of the stainable hepatic "glycogen" content at the time of death. Chemical analysis for glycogen, however, shows marked decreases during the same period.

The discrepancy in the histologic evaluation and chemical determination of "glycogen" is not explained. The situation is all the more puzzling because the histologic stains for glycogen give parallel results among themselves, as do also the various quantitative chemical procedures.

We wish to express our apprecation to Miss Evelyn Paget for her excellent histologic preparations.

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DESCRIPTION OF PLATES

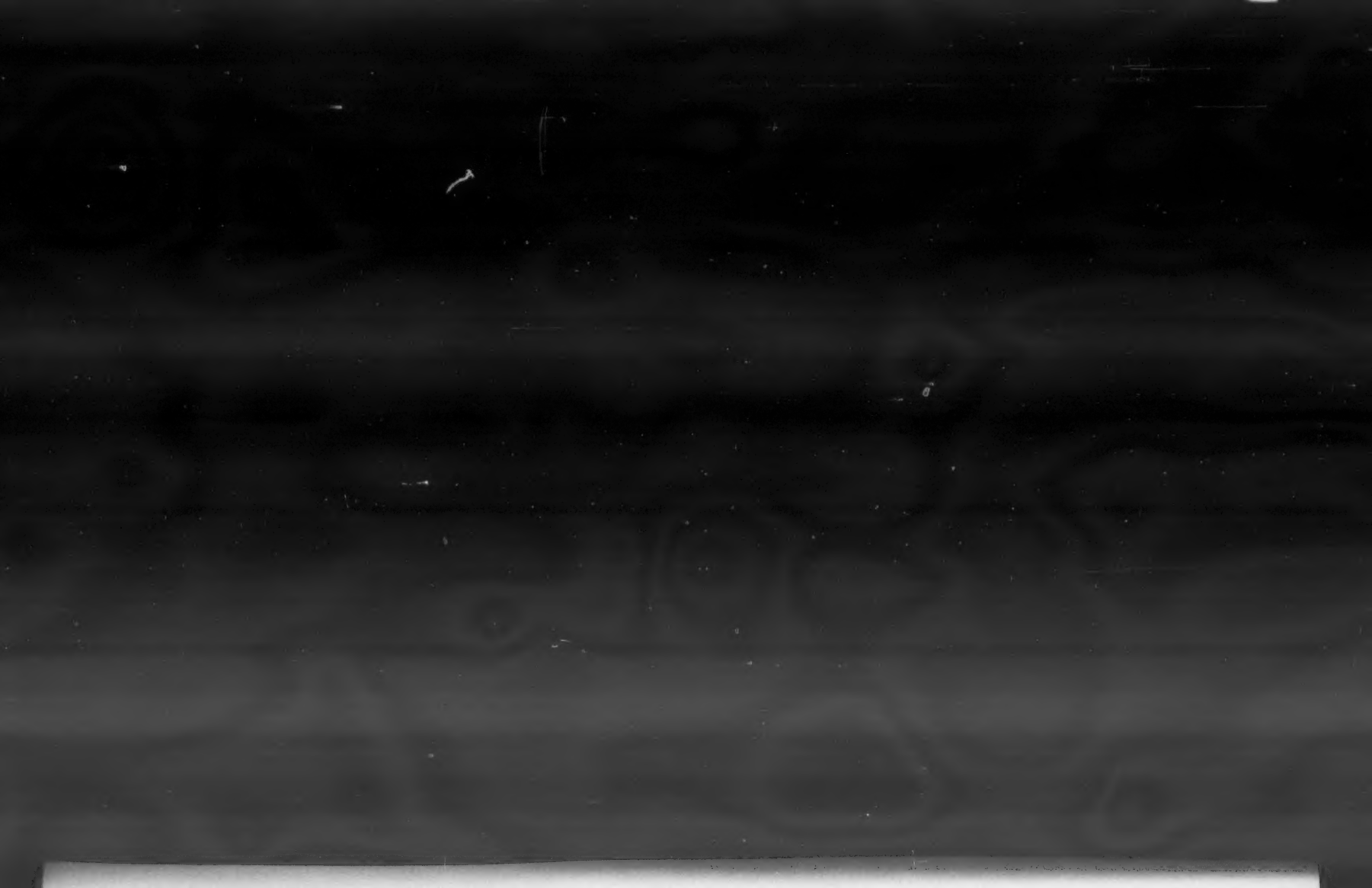
PLATE 79

FIG. 1. Rat liver fixed immediately after death in 10 per cent formalin in 95 per cent alcohol. Glycogen content, 6.4 per cent. Lillie's modification of the Bauer stain.[1] × 185.

FIG. 2. Same liver as shown in Figure 1, fixed 4 hours after death in alcoholic formalin. Glycogen content, 3.8 per cent. Lillie's modification of the Bauer stain. × 185.

FIG. 3. Same liver as shown in Figures 1 and 2, fixed 24 hours after death in alcoholic formalin. Glycogen content, 1.2 per cent. Lillie's modification of the Bauer stain. × 185.

FIG. 4. Rat liver fixed 24 hours after death in alcoholic formalin. Glycogen content, 0.82 per cent. Lillie's modification of the Bauer stain. × 230.



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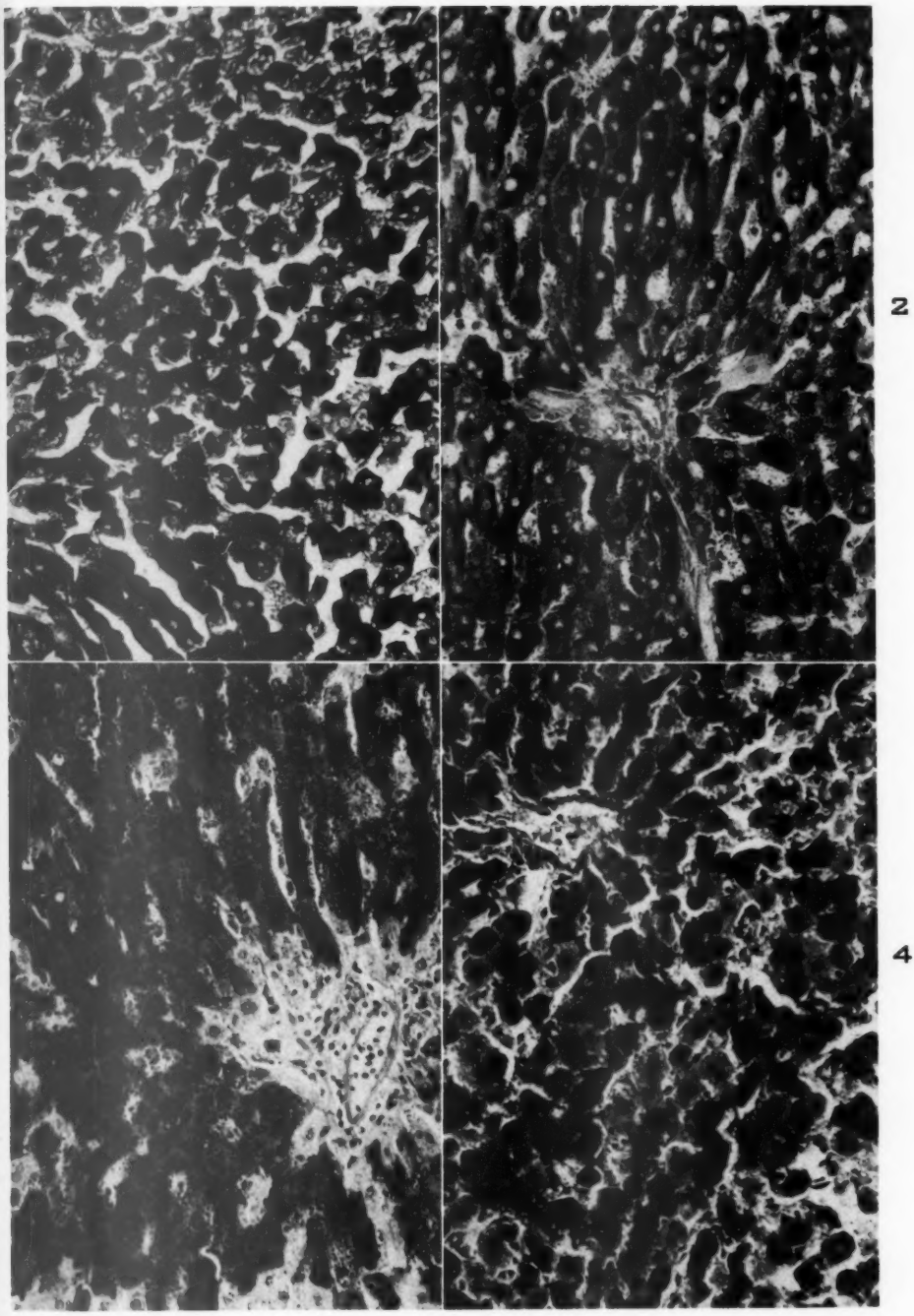

Morrione and Mamelok Hepatic Glycogen after Death

PLATE 80

FIG. 5. Human liver removed for biopsy in a case of obstructive biliary cirrhosis. Fixed in alcoholic formalin 24 hours after removal. Large amounts of glycogen persist. Lillie's modification of the Bauer stain. × 250.

FIG. 6. Human liver removed for biopsy in a case of cirrhosis with primary hepatic cell carcinoma. Fixed in alcoholic formalin 24 hours after removal. The tumor cells show as much glycogen as did a specimen fixed immediately after removal. Lillie's modification of the Bauer stain. × 300.

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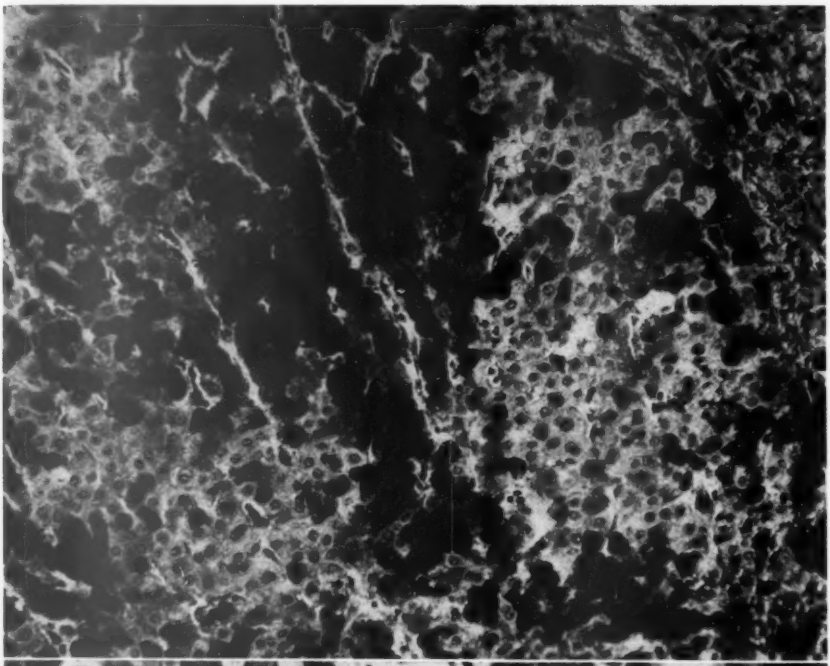

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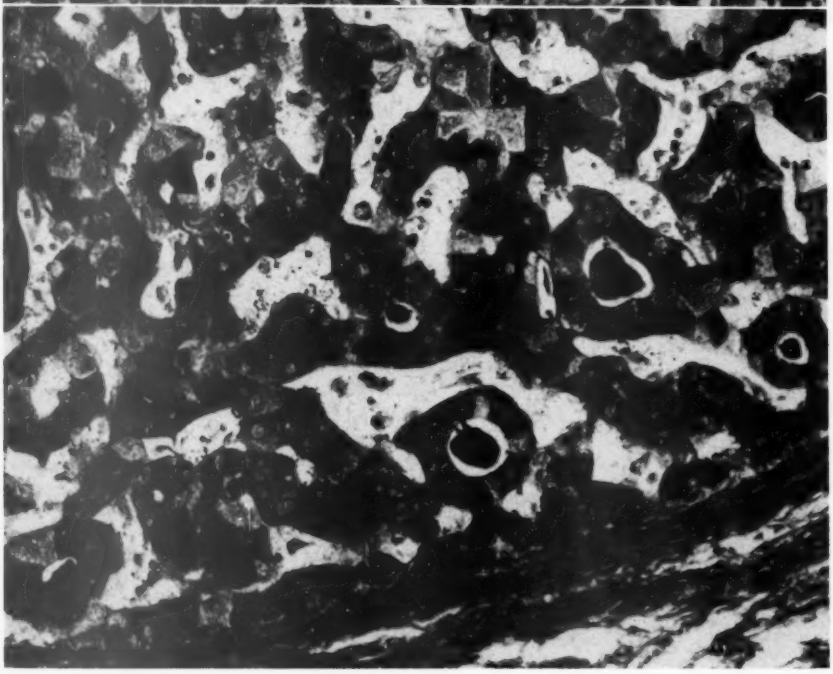

Morrione and Mamelok Hepatic Glycogen after Death

FORTY-NINTH ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS

AND BACTERIOLOGISTS

NEW YORK CITY

APRIL 10TH, 11TH, AND 12TH, 1952

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-Ninth Annual Meeting,
Hotel New Yorker,
New York, New York
April 10th, 11th, and 12th, 1952

PRESIDENT MOORE IN THE CHAIR

BUSINESS MEETING

April 10, 1952

Upon nomination of the Council, the Association elected the following officers:

President	DR. WILLIAM H. FELDMAN
Vice-President	DR. JAMES B. MCNAUGHT
Secretary	DR. ALAN R. MORITZ
Treasurer	DR. SIDNEY FARBER
Incoming Member of Council	DR. GRANVILLE A. BENNETT

The President announced that the Council had elected Dr. Paul Klemperer as a member of the Council to fill the unexpired term of Dr. Tracy B. Mallory.

The President announced that the following non-voting officers had been elected by the Council:

Assistant Secretary	DR. J. LOWELL ORBISON
Assistant Treasurer	DR. WILLIAM A. MEISSNER

For the Council, the President announced the following actions:

Election of New Members

James D. Barger, Phoenix, Ariz.
Frederick C. Bauer, Jr., Chicago, Ill.
Nolton H. Bigelow, Albany, N.Y.
Boyd K. Black, Bethesda, Md.
Robert L. Breckenridge, Philadelphia, Pa.
William P. Callahan, Jr., Wichita, Kans.
William T. Collins, Cincinnati, Ohio
Charles C. Congdon, Bethesda, Md.
Zola K. Cooper, St. Louis, Mo.
Clyde G. Culbertson, Indianapolis, Ind.
Ernst J. Eichwald, Salt Lake City, Utah
Irwin Feigin, New York, N.Y.
Jacob K. Frenkel, Memphis, Tenn.
Frederick G. Germuth, Jr., St. Louis, Mo.
John T. Godwin, Upton, N.Y.
Raymond G. Gottschalk, Houston, Texas
Donald B. Hackel, Cleveland, Ohio

Walter S. Hartroft, Toronto, Canada
Willard T. Hill, Chicago, Ill.
John B. Holyoke, Hanover, N.H.
Robert E. Hoyt, Venice, Calif.
Russell S. Jones, Memphis, Tenn.
Joseph R. Kahn, Shaker Heights, Ohio
Nathan Kaufman, Cleveland, Ohio
Johannes P. Kulka, Boston, Mass.
Baldwin G. Lamson, Los Angeles, Calif.
Joseph F. McCloskey, Philadelphia, Pa.
Chester R. McLean, Toronto, Canada
Norman G. B. McLetchie, Halifax, Canada
Philip O'B. Montgomery, Boston, Mass.
Harold R. Pratt-Thomas, Charleston, W.Va.
Francesco Di Raimondo, Bari, Italy
Charles C. Randall, Nashville, Tenn.
Henry Rappaport, Arlington, Va.
Roderick C. Ross, Montreal, Canada
Louis A. Schneider, Fort Wayne, Ind.
Michael B. Shimkin, San Francisco, Calif.
William M. Silliphant, Washington, D.C.
David E. Smith, St. Louis, Mo.
John Chandler Smith, Chicago, Ill.
George F. Stephenson, Chicago, Ill.
William St. Clair Symmers, Birmingham, England
Harold E. Taylor, Vancouver, Canada
Wilfred E. Toreson, San Francisco, Calif.
Eleanor H. Valentine, Denver, Colo.
Austin L. Vickery, Jr., Boston, Mass.
F. Stephen Vogel, New York, N.Y.
Douglas O. W. Waugh, Montreal, Canada
Robert W. Wissler, Chicago, Ill.
Lewis B. Woolner, Rochester, Minn.

Pearl M. Zeek, Cincinnati, Ohio

Acceptance, with regret, of the resignations of Drs. J. Harold Austin (now deceased), John Kent, David G. Mason, Ralph Muckenfuss, John Sugg, J. S. Taylor, and Cecil J. Watson.

With deep regret, the recording of the deaths of Drs. Francis G. Blake, Chester R. Brown, Carl J. Bucher, Henry A. Christian, George H. Hansmann, Joseph G. Hopkins, Katherine M. Howell, Oscar B. Hunter, Sr., Robert Koritschoner, George M. Mackenzie, Tracy B. Mallory, George W. McCoy, Mark P. Schultz, Malcolm H. Soule, and George A. Snyder.

The re-election of Dr. Howard T. Karsner to the Editorial Board of *The American Journal of Pathology* for a period of six years, and the election of Dr. Ernest M. Hall to fill the unexpired term of Dr. Tracy B. Mallory on the Editorial Board.

The election of Miss Dorothy Seiferlein as Editorial Assistant of *The American Journal of Pathology.*

The nomination of Dr. Edwin W. Schultz to serve on the Board of Trustees of the American Type Culture Collection.

The President announced that the next annual meeting of the Association will be held in St. Louis, Missouri, on April 2, 3 and 4, 1953. The topic for the symposium is "The Modification of Structural Changes in Infectious, Neoplastic, and Other Diseases Following Use of Modern Chemotherapeutic Agents." Drs. Sidney Farber and William H. Feldman have been invited to act as referees.

The President announced that as the result of a postal vote, the Council had agreed to discontinue stenographic recording of discussions at the scientific meetings unless circumstances in future years make it seem desirable.

The following results are recorded for the general postal vote taken of the entire membership of the Association in regard to time and place of holding meetings. Only unqualified votes were included in the count.

Where to hold meetings

83 Favor meeting in universities
333 Favor meeting outside universities

159 Favor meeting in cities where there is a medical school
300 Favor meeting in any city

263 Favor meeting in a hotel
106 Favor meeting in a medical institution

When to hold meetings

163 Favor independent meeting
241 Favor meeting the week preceding the Federation meeting in the same town

227 Favor opening negotiations directed toward formation of a Federation of Societies of Pathologists
201 Favor the present plan

How to arrange the meetings

150 Favor a single program
269 Favor multiple programs on some half days

206 Favor independent meetings (*i.e.*, independent of Society for Cancer Research and Federation)

213 Favor opening negotiations directed toward arrangements for joint meeting

(If joint meeting can be arranged)
242 Favor Saturday morning
47 Favor Saturday afternoon

The meeting was then adjourned.

By postal vote of the Council, the Gold Headed Cane was awarded to Dr. Howard T. Karsner. Presentation was made at the annual dinner, April 10, 1952.

Alan R. Moritz, *Secretary*

REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted. It was accompanied by a letter of certification from Frank D. Flynn, Auditor, Melrose, Massachusetts. In condensed form, the Treasurer's report follows:

General Checking Account

Receipts

Balance on hand, January 1, 1951		$ 2,208.56
Membership dues	$ 7,791.73	
Interest on bonds	500.00	
Interest transferred from savings banks	1,510.56	
		9,802.29
		$12,010.85

Disbursements

American Journal of Pathology ($8.00 per member; final quarter paid in 1952)	$ 4,800.00	
Catherine Lennon (secretary to Dr. Moritz)	250.00	
Mary Sabin (clerical assistant to Dr. Moritz)	100.00	
Harry McGachie (secretary to Dr. Farber)	150.00	
Reporting 1951 meeting	326.96	
Officers' expenses at meeting (including printing, badges, travel)	403.35	
Auditing services	35.00	
General office expense, secretary	176.86	
General office expense, treasurer	66.76	
		6,308.93
Balance on hand, December 31, 1951		$ 5,701.92

Investment Account

Balance, January 1, 1951	$35,016.84	
Interest on bonds, 1951	500.00	
Interest from savings banks	493.72	
		$36,010.56
Transfer to checking account (bond interest)	500.00	
Transfer to checking account (accumulated bank interest)	1,510.56	
		2,010.56
Balance, December 31, 1951		$34,000.00

Inventory

U.S. bonds, series G	$20,000.00
Provident Institute of Savings	4,000.00
Franklin Savings Bank	4,000.00
Cambridge Savings Bank	4,000.00
National Shawmut Bank	2,000.00
Total, December 31, 1951	$34,000.00

Sidney Farber, *Treasurer*

SCIENTIFIC PROCEEDINGS

INTERACTION BETWEEN EASTERN EQUINE ENCEPHALOMYELITIS VIRUS AND CERTAIN RESORCINOL DERIVATIVES. Murray Sanders and (by invitation) Jon J. Kabara, Manuel G. Soret, and Benjamin A. Akin, University of Miami Medical Research Unit, at Veterans Administration Hospital, Coral Gables, Fla.

Abstract. The mutual action between virus protein and organic compounds is of interest to several groups of investigators. The bacteriologist and pathologist must study the phenomena in screening programs for potential antiviral agents. The oncologist, increasingly concerned with virus problems, must pay close attention to both chemical agent and virus, individually, and as a complex. Biochemists find this interaction of importance as a potential model in studying virus activity and nucleoprotein metabolism.

Past investigations of dyes and related chemical agents on virus activity have been limited almost entirely to compounds with basic groups. This report is concerned with acid compounds and is part of a program involving both tumors and viruses. The general method of investigation was the study of the dynamic interaction between compound and virus under controlled conditions of varying pH, temperature, and concentration. Plotting the results for their kinetic interpretation gave a complex reaction representing two or more simple first order reactions. Results to date indicate the following:

1. The entire process of inactivation was essentially completed within 1 hour at 37° C., although virus could be measured for 6 hours at this temperature.

2. Once a virus is inactivated it is avirulent as demonstrated by mouse passage and histopathologic studies.

3. Virus inactivation is influenced by the number of halogen atoms present and their steric position. Furthermore the character of the halogen plays an important rôle in the inactivation. The iodine derivatives seem to be the most active.

The completely inactivated virus-dye complex is being investigated immunologically for comparison with living virus as an antigen.

THE PATHOLOGIC CHANGES PRODUCED IN CHICK EMBRYOS BY YOLK SAC INOCULATION OF GROUP A COXSACKIE VIRUS. James H. Peers and (by invitation) Sara E. Ransom and Robert J. Huebner, National Institutes of Health, Bethesda, Md.

Abstract. A series of embryonated eggs ranging in maturity from 6 to 18 days' incubation have been successfully infected by yolk sac inoculation with a strain of group A Coxsackie virus, and the resulting pathologic changes have been described in detail. The principal lesion consisted of widespread acute necrosis of striated muscle, apparently essentially identical in type and development with that characteristically produced by this virus in suckling mice. The infection is not directly lethal to the embryo chicken. Some were found living with virtually complete destruction of skeletal muscle, apparently because muscle was not required for either respiratory or nutritional activity before hatching. Increasing maturity of the embryo at the time of inoculation lessened the severity and extent of the resulting lesions, but did not, up to the 18th day of incubation, abolish susceptibility to infection. The lessening of severity of the lesions is especially marked and abrupt between embryos inoculated on the 10th and the 12th day of incubation. Two embryos inoculated on the 18th day of incubation hatched and lived for 4 and 11 days respectively. The first showed minimal specific muscle lesions, but the second only some irregularity in muscle fiber size.

In addition to the muscle lesions characteristic of infection with group A Coxsackie virus, there were regularly present in the more immature embryos extensive necrosis of epidermis and feathers, and degeneration of feather follicles believed to be probably a direct dermotropic effect of the virus. Other visceral lesions, chiefly liver

necrosis, were observed occasionally but were believed not to be due to direct virus action. As in suckling mice infected with group A Coxsackie virus, no lesions were observed in the central or peripheral nervous system nor in the heart muscle. No lesions considered to be specific were seen in the few specimens of chorioallantoic membranes examined.

THE PATHOLOGY OF CORTISONE-ENHANCED POLIOMYELITIS IN THE HAMSTER FOLLOWING INTRACEREBRAL INOCULATION OF STRAIN MEF_1. Stanley M. Aronson (by invitation) and Gregory Shwartzman, Mt. Sinai Hospital, New York, N.Y.

Abstract. It has been shown previously by one of the authors (G. S.) that intramuscular administration of cortisone concurrently with the intracerebral inoculation of poliomyelitis virus significantly enhances the resultant disease in the hamster. In the following studies on the pathogenesis of the infection modified by means of cortisone, the hamsters were sacrificed daily and all tissues, cords, and serially sectioned brains were examined histologically.

Clinically, hamsters receiving cortisone showed a 95 per cent incidence of paralysis (in contrast to 50 per cent in the controls), presented their paralytic symptomatology at an earlier date, and uniformly succumbed to the disease. The control group of hamsters, receiving only intracerebral virus, when developing paralysis, showed a more torpid clinical course, a lesser tendency toward bilateral limb involvement, and a higher percentage of clinical recovery.

A number of striking histologic differences were apparent. In the control group, there was seen a prompt cellular and vascular reaction to the needle tract injury. A polymorphonuclear leukocyte response was visible in 18 hours, endothelial hyperplasia was conspicuous by the second day, and microglial activation apparent by the third day. These defense mechanisms were totally inapparent in the cortisone-treated group during the initial days of infection. The control group usually produced a topographically limited viral infection reflected morphologically by minimal neuronal involvement, inability to involve contralateral nuclear groups, and, in contrast, an extremely exuberant interstitial inflammatory and microglial reaction as well as pronounced perivascular accumulations. In those instances, in the control group, wherein the infection spread to the anterior spinal horns, the intervening medulla was only minimally affected by the process. The group which received cortisone, on the other hand, failed to manifest the usual central nervous system response to viral infection until the fifth or sixth day, and even then in a subdued fashion. Neuronal destruction, however, was dramatically widespread, invariably bilateral, and observable at an earlier date than in the control group. The pathologic landmarks of poliomyelitis (*i.e.*, neuronophagia, inflammatory cell aggregation, and perivascular cuffing) were conspicuously absent until very late in the course of the infection.

Thus, one of the factors responsible for the potentiation of poliomyelitis by cortisone may reside in the ability of this hormone to inhibit significantly the normal central nervous system defensive responses of microglial stimulation and phagocytosis, vascular hyperplasia, and mobilization of intravascular inflammatory cells.

MYOCARDITIS IN ASSOCIATION WITH VARICELLA. Donald B. Hackel (by invitation), Cleveland City Hospital and Western Reserve University, Cleveland, Ohio.

Abstract. Numerous recent reports have stressed the relatively high incidence of myocarditis in infectious diseases. Virus diseases, especially poliomyelitis, have been shown to be associated with inflammatory lesions of the myocardium. Nevertheless, a review of the literature failed to reveal previous reports of myocarditis occurring in patients with varicella.

Of 11,550 necropsies performed at the Cleveland City Hospital between 1932 and 1952, 8 patients had varicella at the time of death. Two of these, who showed marked interstitial myocarditis, were excluded from this study because of the coexistence of diphtheria. Myocarditis of slight to marked degree was found in all of the

remaining 6 cases. The lesions were focal and in several cases were not discovered until numerous sections had been studied. They consisted of edema and interstitial infiltration of mononuclear leukocytes, polymorphonuclear neutrophils, and eosinophils. The myocardial fibers were intact for the most part, but a few, in association with some of the inflammatory foci, showed evidence of degeneration, with swelling, loss of striations, and necrosis. No intracytoplasmic or intranuclear inclusion bodies were seen in any case. In 2 cases there was a moderate epicarditis, with infiltration of lymphocytes, plasma cells, polymorphonuclear neutrophils, and eosinophils. In the remaining 4 cases there was a slight epicarditis, with scattered plasma cells and lymphocytes.

All 6 cases had pulmonary involvement, varying from slight interstitial pneumonitis to a moderately severe bronchopneumonia. Two of the cases had a bleeding tendency and one patient had a ruptured appendix. In none of the cases was there any clinical suggestion of myocarditis, which is consistent with the mild and focal character of the myocardial lesions. Although the presence of myocardial lesions in all 6 cases suggests that the varicella virus is the etiologic agent, this cannot be considered as proved without isolation of the virus from the myocardium and its use in the experimental production of similar lesions.

THE PATHOLOGY OF EPIDEMIC HEMORRHAGIC FEVER. Robert L. Hullinghorst (by invitation), 406th Medical General Laboratory in Tokyo, Japan.

Abstract. The disease epidemic hemorrhagic fever which has been recognized in parts of Manchuria and localized areas of the USSR, has been studied by Russians and Japanese for the past 2 decades, but was largely unknown to American medicine until it became epidemic in UN forces in Korea in 1951. The disease is thought to be due to a virus harbored in field rodents and transmitted to man by an arthropod vector. Clinically it consists primarily of an acute fever, a marked leukemoid reaction, widespread hemorrhage, and severe renal damage. The large swollen kidney with hemorrhagic pyramids and peripelvic hemorrhage is felt to be most characteristic. There are three general morphologic abnormalities seen microscopically. They are (a) focal necrosis suggesting infarction, seen in decreasing order of frequency in kidney, pituitary and adrenal glands, pancreas, and liver; (b) marked engorgement, increased permeability, and rupture of capillaries; and (c) an inflammatory reaction consisting chiefly of proliferation of tissue macrophages and infiltration of immature leukocytes. Strangely enough, areas of hemorrhage and necrosis do not appear to be the prime excitants of this reaction. Focal myocarditis and endocarditis are common. The heart is usually also the site of numerous hemorrhages, particularly in the wall of the right atrium. In fatal cases death is usually due to shock in the early stages, and to uremia later.

LIVER BIOPSIES IN INFECTIOUS MONONUCLEOSIS. Richard C. Wadsworth and Philip G. Keil (by invitation), Eastern Maine General Hospital, Bangor, Me., and 132nd Med. Gp. AEB, Dow Field.

Abstract. Because of the relatively benign character of infectious mononucleosis, there has been a very limited opportunity, from the study of post-mortem material, to ascertain the anatomical effects of this disease. There are in the literature reports of 21 necropsies on patients who died either during or shortly after an attack of infectious mononucleosis. Most of these reports agree as to the widespread effects of this disease. Although numerous articles have appeared in the literature concerning liver dysfunction in non-fatal cases of infectious mononucleosis, most of these reports have dealt with the abnormal results obtained by various liver function tests. Fourteen liver biopsies have been reported in 8 different publications. The largest reported series included 5 liver biopsies.

During the past 6 months 12 liver biopsies were obtained from 10 cases of infectious mononucleosis. Serial liver function studies were obtained on most of these

patients in an attempt to correlate the histologic findings with the clinical course and laboratory findings. Biopsies were taken from 1 to 30 weeks after the onset of symptoms. All biopsies were taken with forceps under peritoneoscopy. The pathologic findings are quite similar to those described by Mallory in cases of non-fatal epidemic hepatitis. The most marked abnormalities, in order of prominence, are mobilization of Kupffer cells, periportal cellularity, nuclear variation, degeneration and necrosis of liver cells, and slight bile duct reduplication. Mitotic activity in liver cells is not marked. The degree of anatomical change does not parallel the degree of abnormality of the liver function tests.

EXTRAHEPATIC OBSTRUCTIVE JAUNDICE IN LIVER BIOPSY SPECIMENS. Paul B. Szanto, Hans Popper, and (by invitation) Lawrence J. Manuell, Hektoen Institute for Medical Research, Cook County Hospital, Chicago, Ill.

Abstract. The histologic alterations in obstructive jaundice, as seen in liver biopsy specimens, are of diagnostic importance for possible differentiation from primary hepatitis. Therefore 110 biopsy specimens of 100 patients suffering from various types of biliary obstruction were studied, with 100 necropsy specimens of similar cases of obstruction and 110 biopsy specimens of primary hepatitis for comparison. The morphologic findings resulting from extrahepatic biliary obstruction depend upon the duration and degree of obstruction and upon the presence of complicating infection. The following changes assist in the differential diagnosis between extrahepatic obstruction and primary hepatitis: (1) Some evidence of hepatocellular degeneration, characterized by variation in size and shape of the nuclei within the same zone, appears early after the onset of the obstruction. Regeneration and irregular arrangement of the liver cell plates are uncommon in contrast to primary hepatitis. Binucleated and multinucleated liver cells with large nucleoli are less frequent than in other types of hepatic injury. (2) Prolonged obstruction leads to three types of liver cell necrosis: (a) necrosis of isolated bile-imbibed hepatic cells in the central zone; (b) formation of "bile lakes" due to disappearance of liver cells around the bile plugs; (c) reticular necrosis of bile-imbibed liver cells in the peripheral zone with connective tissue replacement. (3) The mobilized Kupffer cells seem to contain a large amount of bile pigment and less basophilic material in the cytoplasm than in other types of hepatic injury. If bacterial infection complicates extrahepatic obstruction, some of the mobilized Kupffer cells become rich in cytoplasmic basophilic material. After decrease or disappearance of biliary obstruction the Kupffer cells contain bile pigment much longer than the bile capillaries. (4) The presence of bile casts (microcalculi) in dilated bile ducts is a characteristic feature of extrahepatic obstruction. Only after prolonged obstruction does necrobiosis occur in the lining epithelium with subsequent rupture and bile extravasation. The other changes in the portal triads like edema, concentric fibrosis, and proliferation of ducts and ductules are not specific. The fibrosis does not cause significant lobular distortion. Bacterial infection superimposed upon extrahepatic obstruction, usually reflected in abnormal flocculation reactions, is mirrored in the accumulation of segmented leukocytes around or within ducts and ductules. The inflammatory exudate accentuates the fibrosis in the portal triads with a subsequent tendency to distortion of the lobular pattern. Attempts are being made to develop morphologic criteria for differentiation between extrahepatic and intrahepatic ("cholangiolitic") obstruction.

HEPATIC AND PANCREATIC CHANGES PRODUCED IN RATS BY ETHIONINE AND THEIR RELATION TO HUMAN LESIONS. Hans Popper and (by invitation) J. de la Huerga and Dieter Koch-Weser, Hektoen Institute for Medical Research, Cook County Hospital, Chicago, Ill.

Abstract. Tissue changes produced by ethionine are presented because of their relationship to disturbances of protein metabolism and because they simulate human

lesions. This permits tracing from an initial non-characteristic stage to a fully developed lesion. Ethionine, an analogue of methionine, interferes presumably with protein synthesis. It produced, within 48 hours, fatty livers in female rats and acute pancreatitis in rats of both sexes. These lesions were considered as the results of competitive interference with methionine utilization and were prevented by simultaneous administration of methionine. Ethionine feeding for 4 weeks produced degeneration and atrophy of pancreatic acinar cells accompanied by interstitial infiltration and fibrosis and followed by abnormal regeneration. The islet cells appear unchanged. Even the chronic pancreatic lesion can be rapidly cured by methionine. This illustrates the importance of protein deficiency as a cause of pancreatitis.

After ethionine administration for 4 weeks diffuse degenerative changes of the liver cells were noted, associated with irregular regeneration and extensive intralobular connective tissue proliferation, diffuse infiltration by reticulo-endothelial cells, and proliferation of cholangioles. The lobular architecture is rarely distorted by thick connective tissue septa. Serum bilirubin, urea, and non-protein nitrogen are elevated; esterase is lowered; blood sugar is normal, while hepatic proteins and lipids are reduced. Study of this lesion in rats on ethionine-containing diets for shorter periods indicates that an initial fatty metamorphosis is followed by central necrosis and diffuse degeneration of hepatic cells. Simultaneously, cholangioles proliferate and along intralobular cholangioles exudative cells accumulate in streaks throughout the lobule. This is followed by new formation of reticulum and subsequently collagenous fibers, resulting in separation of single liver cells or groups of them. The lesion thus simulates human pericholangiolitis and finally cholangiolitic (Hanot) cirrhosis associated with hepatocellular degeneration. A methionine-rich diet cured even far advanced lesions except for some intralobular pericholangiolitic exudate and fibrosis. Subsequent feeding of ethionine restored the lesion leading to bizarre cholangiolar regeneration. The simultaneous appearance and disappearance of pericholangiolitic and hepatocellular changes (also encountered in human lesions) speak for a mutual inter-relationship. The ethionine lesion represents an experimental diffuse hepatic injury (different from the common nutritional cirrhosis) which can be produced, cured, and reproduced at will. It differs from hepatic lesions produced by other amino acid deficiencies.

A Form of Hepatitis in the Neonatal Period Simulating Biliary Atresia. J. M. Craig and B. H. Landing, Children's Medical Center, and the Children's Cancer Research Foundation, Boston, Mass.

Abstract. A review of the liver biopsies performed at the Children's Medical Center over the past 10 years yielded 71 specimens. A striking feature of 11 of these was the presence of very large numbers of giant multinucleate parenchymal cells. These 11 specimens also showed a group of other distinctive features. The general architecture was preserved but disruption of the liver cell cords was very marked. The portal triads were only minimally widened and proliferation of the bile ducts was unusual. Bile stasis in the portal triads was present in but a single case. Infiltration of the portal triads by lymphocytes, eosinophils, and polymorphonuclear cells was moderate in degree. In several cases myelopoiesis was a prominent feature in the portal areas. The distribution of the multinucleate liver cells was nearly uniform throughout the lobules with a slight tendency to an increased frequency about the central vein. The giant cells were often 400 μ in greatest diameter and contained twenty or more nuclei. Their cytoplasm was abundant, granular, and often contained much glycogen. Areas of eosinophilic degeneration could be seen within the cytoplasm. The nuclei frequently showed karyorrhectic and less often pyknotic changes. No mitotic figures were seen in the multinucleate cells and rarely elsewhere. Completely necrobiotic cells were uncommon, and areas of necrosis less so. Bile stasis was widespread within the giant cell forms, but somewhat less frequent in

the canaliculi. The Kupffer cells were swollen and contained much bile pigment. Frequent areas of erythropoiesis were present within the sinusoids, with foci of both erythroblasts and normoblasts. Inflammatory cells were not conspicuous within the lobules, though a few cases showed an acute central vasculitis. Focal areas of condensation of the reticulum were present.

An examination of the clinical records revealed that the material having the above characteristics came from infants 2 months of age and younger, in 5 of whom jaundice had developed during the first day of life, in 4 during the first week, and in the remainder during the first 2 months. All developed dark urine, acholic stools, and elevated direct and indirect serum bilirubins. The liver function tests, when performed, indicated an obstructive type of jaundice. The findings suggested biliary atresia. Exploratory laparotomy revealed a normal and patent extrahepatic bile duct system. In 2 of these cases inspissated secretions were encountered in the gallbladder. Postoperative follow-up revealed that 4 are now well, 1 has continuing jaundice and liver failure, 1 has died with liver failure, and the remainder are too recently postoperative, or no follow-up has been possible.

In addition, 5 cases from the necropsy files are included, all of which had similar findings. There is a striking similarity between the histologic features in these cases and those in liver biopsy specimens of epidemic hepatitis.

STUDIES ON THE REDUCTION OF TETRAZOLIUM DERIVATIVES (TD) BY NORMAL AND DISEASED HUMAN TISSUES. Y. T. Hsu (by invitation) and Cornelia Hoch-Ligeti (by invitation), University of Virginia School of Medicine, Charlottesville, Va.

Abstract. The enzymatic reduction of the colorless, water-soluble triphenyltetrazolium chloride (TTC) to a red, water-insoluble formazan has been described to differ in malignant and normal tissues. Some authors considered the differences significant enough to recommend their use for diagnostic purposes in tissue samples as well as in living persons. Other authors, however, failed to confirm the differential enzymatic reduction of TD in malignant tissues. To evaluate the possibility of using TD for the diagnosis of malignancy in human tissues the reduction of TTC and neotetrazolium in over a hundred operative specimens was investigated. As a rule only a few minutes elapsed between the removal of the tissue and its incubation with TD. Slices thicker than about 0.2 to 0.5 mm. were not stained uniformly. Frozen sections 8 to 10 μ thick, incubated without added substrate, did not reduce TD, but reduction occurred when sodium succinate was added to the incubating fluid. TTC and neotetrazolium behaved in a similar fashion and are discussed together.

Normal connective tissues did not reduce TD. Fatty tissues stained pink, but as reduced TD is soluble in fat, it could not be decided whether the staining was due to enzymatic activity. Striated and smooth muscle were weakly active. Endothelium of vessels was very weakly stained or remained unstained. Epithelial cells contained always reductase, glandular epithelium being more active than squamous epithelium. Kidney and liver showed strong activity, the distribution in the liver cells was uniform; in kidneys the convoluted tubules showed the strongest activity. Glomeruli were negative, collecting tubules weakly stained. In benign tumors and in chronic inflammation, the participating tissues behaved like homologous normal tissue, *i.e.*, connective tissue was inactive, epithelium active. Epithelioid cells in tubercles reduced TD.

Epithelial cells in all carcinomas showed reductive activity, sometimes weaker, sometimes stronger than the surrounding normal epithelium or epithelium of benign tumors. Sarcomas and the stroma of carcinomas repeatedly, but not always, reduced TD, and in that they differed from all normal adult connective tissues. Whether the reduction of TD by the fibrous elements in malignant tissues is due to a pathologic

change or is the expression of rapid growth will be investigated further. Due to the infiltration of tumor cells into the surrounding tissues and the staining of the stroma, malignant tumors appeared to the naked eye more diffusely stained than benign tumors. From the series of investigations it seems unlikely that the reduction of TD in operative material could be used for the diagnosis of cancer.

NEOTETRAZOLIUM AS A HISTOCHEMICAL INDEX OF CELL METABOLISM IN THE OVARY. Alvan G. Foraker and (by invitation) Sam W. Denham, Emory University School of Medicine, Atlanta, Ga.

Abstract. Tetrazolium salts as indicators of dehydrogenase activity are new additions to the histochemical armamentarium. An insoluble formazan is said to be formed at the sites of active metabolic processes. In order to test the pattern of formazan deposition in relation to cell metabolism, a series of ovaries from rabbits undergoing Friedman tests was processed in neotetrazolium. Ovaries from all rabbits revealed diffuse blue-black granular deposition through the stroma, more about the vessels, and with heavier deposition in the follicular cells about the numerous ova. The ova contained little formazan. Numerous cystic follicles were found in the ovaries of rabbits injected with urine from non-pregnant women. The granulosa cell layer of these generally had a deposition of small formazan granules, usually somewhat more concentrated than in the adjacent stroma. In the ovaries of rabbits injected with urine from pregnant women, the cell layers of the corpora hemorrhagic and corpora lutea usually contained much heavier formazan deposits with larger granules than seen elsewhere. Cell layers adjacent to some of the largest hemorrhages occasionally had little or no formazan deposition. This may result from:

1. Chemical interference by the blood in the hemorrhagic follicle.
2. Diminution of cellular metabolism of adjacent tissue once massive hemorrhage has occurred.

The pattern and degree of formazan deposition corresponded to anticipated areas of intense cellular metabolism in the ovary.

OBSERVATIONS ON THE GOMORI SUBSTANCE IN THE HYPOTHALAMUS OF DOGS AND RATS UNDER NORMAL AND EXPERIMENTAL CONDITIONS. G. L. Laqueur, National Institutes of Health, Bethesda, Md.

Abstract. Serial sections of hypothalami of dogs and rats were stained with Gomori's chromium-hematoxylin-phloxine method according to Bargmann. Alternate sections were stained with toluidine blue for the demonstration of Nissl substance and with the periodic acid-Schiff procedure for the study of the vascular system. Gomori-positive granules were found in normal dogs within the ganglion cells of the supra-optic, paraventricular, and infundibular nuclei, and along the course of the fiber tracts extending from these nuclei into the posterior lobe of the hypophysis. Large amounts of Gomori-positive material were commonly associated with an apparent reduction in the Nissl substance. Daily injections of 100 mg. of cortisone over 14, 44, and 71 days respectively did not alter either the ganglion cells or the amount of Gomori-positive material. Identical methods when applied to normal adult rats demonstrated only minute amounts of Gomori-positive material in 2 of 7 animals. However, removal of the posterior lobe or total hypophysectomy resulted in an accumulation of Gomori-positive substance in the hypothalamic nuclei.

Adrenalectomized rats, maintained on 0.9 per cent NaCl, did not show Gomori-positive granules in the hypothalamus. However, such material was observed in adrenalectomized rats which had been injected daily with 2.5 mg. of cortisone for 12 days. Dehydration, induced by withholding water or by the injection of a 5 per cent solution of NaCl, resulted in reduction of Nissl substance and appearance of stainable granules during the first 12 hours of the experiment. Prolonged dehydration

(96 hours) was associated with large amounts of Nissl substance in the cells of the paraventricular and supra-optic nuclei and complete absence of Gomori-positive granules.

The present status of the nature of the Gomori substance in the hypothalamus, its localization, and its possible significance with respect to factors controlling water metabolism were summarized briefly.

A Histochemical Study of the Distribution and Fate of Dextran in Tissues of the Mouse. Robert W. Mowry and (by invitation) R. Carl Millican, National Institutes of Health, Bethesda, Md.

Abstract. Dextran is used as a plasma substitute in the treatment of shock. Excretion studies show that about one-third of intravenously injected dextran appears in the urine. The fate of the remaining dextran is in doubt. Methods for the histochemical demonstration of dextran were recently reported by us. Additional work has resulted in a more sensitive and simpler histochemical method that has been applied in the present study. Clinical dextrans of both American and Swedish manufacture were injected intravenously as 6 per cent solutions in 0.9 per cent NaCl. Tissues were fixed and sections cut without exposure to water. For staining of dextran, sections were oxidized for 2 hours in 1 per cent periodic acid in 90 per cent alcohol, rinsed, treated for 10 minutes in Schiff reagent, and taken through bisulfite rinses. Duplicate sections were stained by the aqueous periodic-Schiff routine. Dextran was purplish-black after alcoholic periodic acid-Schiff staining, but was dissolved and therefore uncolored by aqueous periodic-Schiff staining.

Experimental. 1. Tissues of 21 mice were taken at intervals of 2 to 96 hours after a single 1 ml. injection of dextran. At 2 hours, histochemically demonstrable dextran was present in all blood vessels, in liver cells, and in renal tubules. By 4 hours, dextran was also visible in Kupffer cells. In succeeding specimens, increasing amounts of dextran were seen not only in liver cells and Kupffer cells, but also in the red pulp of the spleen and in numerous phagocytes of lung, pancreas, and kidney.

2. Thirteen mice were each given a total of 9 ml. of dextran over a period of 2 weeks and sacrificed from 5 hours to 3 months after completion of injections. Much greater amounts of dextran were found in the previously mentioned sites. Study of other tissues showed numerous dextran-laden phagocytes in lymph nodes, myocardium, and adipose tissues. Dextran was still present in most sites after 3 months but was reduced in amount.

3. Three groups of 6 mice each were given 1, 2, and 3 ml. of dextran respectively. Two mice from each group were examined at 1 month, 2 months, and 3 months. At each postinjection interval the amount of demonstrable dextran appeared proportional to the dose. Dextran was still present in phagocytes of skin and other tissues as long as 3 months after only 1 ml. of dextran. At each level of dosage there was a moderate reduction in demonstrable dextran with increasing postinjection interval.

4. Naturally occurring dextran-like material was not found in similar tissues identically examined from 20 mice not given dextran.

The methods permit the intracellular localization of intravenously injected dextran in a variety of tissues for a considerable length of time. Specifically, dextran has been demonstrated in liver cells, renal tubules, and in widely distributed phagocytes composing the reticulo-endothelial or macrophage system.

Effects of Tissue Extracts and of Protein-split Products upon Leukocytes and upon Capillary Permeability. George A. Tershakovec (by invitation) and Virgil H. Moon, University of Miami, Coral Gables, Fla.

Abstract. Recently we have reported a technic for demonstrating chemotaxis by means of capillary tubes. This method has the advantages of relative simplicity and of providing numerical data. We have shown that watery extracts of various normal

tissues attract leukocytes into capillary tubes against the force of gravity. This property varies in different tissues; it is stronger in lung, skin, and kidney, and is weaker in muscle, liver, and testes. Various products of protein cleavage were tested for chemotactic properties by this technic. Extract of beef, N. F., 4 per cent solution adjusted to pH 7.0, attracted leukocytes, but the degree of chemotaxis was less than that of an extract of fresh tissues. Meat peptone, dry (Fisher), and proteose peptone, neo-peptone, bacto-peptone and bacto-proteose (each by Difco) were moderately chemotactic; the degree of this also was less than that of fresh tissue extracts.

One to 2 ml. of 1 per cent solution of trypan blue was given intravenously to rabbits and rats. Then a minute amount of tissue extract was injected into the skin. An area 1.0 to 1.5 cm. in diameter surrounding the point of injection promptly developed a deep blue color. The same results followed intradermal injections of beef extract and of the products of protein cleavage mentioned. These results indicate increased capillary permeability in the affected areas.

Our data indicate that watery extracts of tissues, also various products of protein cleavage, attract leukocytes *in vitro* and cause increased capillary permeability *in vivo*. These phenomena are related to the dynamics of acute inflammation.

DOES BRUCELLOSIS CAUSE CALCIFIC AORTIC STENOSIS? II. THE PATHOLOGIC EVIDENCE. Thomas M. Peery, George Washington University School of Medicine, and George Washington University Hospital, Washington, D. C.

Abstract. Among the various disorders of the cardiac valves, calcific aortic stenosis is unique in the degree of nodularity, deformity, and calcification that occurs. Probably for this reason, and because the disorder is much more frequent in males than in females, this type of heart disease has been set aside in a class by itself. In both these features it differs from disease of the cardiac valves due to rheumatic fever. Yet, in a significant proportion of the cases, there is a history of "rheumatism" with migratory polyarthritis and fever. Several observers have suggested that calcific aortic stenosis is the end result of healing in bacterial endocarditis. However, no one has pointed to a particular type of endocarditis that would explain the clinical and pathologic features.

Brucella infection is suggested as the cause of calcific aortic stenosis. Brucellosis is a febrile disease in which polyarthritis is common and which is frequently confused with rheumatic fever. Males are affected about four times as frequently as are females, corresponding with the sex incidence of calcific aortic stenosis. Furthermore, the granulomas of brucellosis, as they occur in the joints and lungs, are known to heal by fibrosis and calcification. Brucellosis is known to cause endocarditis of a chronic type with nodularity, deformity, and calcification. Many patients dying during active brucellosis show bacterial endocarditis at necropsy. In brucella endocarditis the aortic valve is involved much more frequently than are the other heart valves. A small group of cases tending to confirm this concept is reported.

EXPERIMENTAL ENDOCARDITIS AND OTHER CHANGES IN DOGS WITH ARTERIOVENOUS FISTULAS AND BACTEREMIA. J. R. R. Bobb (by invitation), E. E. Aegerter, and T. G. Anderson (by invitation), Temple University School of Medicine, Philadelphia, Pa.

Abstract. Dogs with large arteriovenous fistulas have been found to be exquisitely susceptible to bacterial endocarditis. This accidental finding led to experiments in which bacteremia and endocarditis were induced in fistulous dogs by injecting relatively small numbers of bacteria. These animals usually died a few weeks after the bacteria were injected. Accompanying the typical lesions of bacterial endocarditis, glomerulonephritis and "rheumatic-like" lesions on the heart valves were described by Bell and Clawson.

Since these diseases in the human being are usually of a subacute, chronic, or recurrent nature, an experiment was designed to investigate the changes which would be produced in animals with longer exposure to the trauma of fistulas and bacteremia. Large arteriovenous fistulas (bilateral iliac, iliac-femoral, or single iliac) were produced. The dogs were then injected daily for a week with 0.5 cc. of a 24-hour broth culture of a group A or D beta hemolytic streptococcus given intravenously. After a bacteremia was established, as shown by repeatedly positive blood cultures, the dog's clinical course was carefully followed. The temperature, heart rate, body weight, and general condition were noted daily. It had previously been observed that the usual course terminally was a persistently elevated temperature, an increased heart rate, a progressive decrease in body weight, anorexia and marked lethargy. At this point antibiotic therapy was started in this series. All of the group A streptococcus infections were cured by twelve injections of penicillin in oil (300,000 units daily). The group D streptococcus was unresponsive to antibiotic therapy and caused the death of all of the animals in which it was used, with the exception of one animal which recovered without any treatment. This organism was most potent in producing a bacteremia. It brought about the death of 5 young single iliac animals which had previously been injected with a group A streptococcus without result. It had been observed previously that young single iliac animals had high resistance to bacterial endocarditis. Over a period of months as many as three periods of persistent bacteremia (each lasting at least several weeks) were induced. All animals, except one, required an antibiotic for cure or died of heart failure or septicemia.

Complete necropsies were performed on all animals. The hearts had hypertrophied ventricles. The aortic and mitral valves showed perforations and fungating lesions. The lungs frequently showed infarcts of varying age and in the dogs dying in heart failure were liver-like, exuding fluid when cut. The abdomen often yielded large quantities (several liters) of ascitic fluid. In one animal the kidney surface had a "flea-bitten" appearance. On examination the microscopic sections disclosed changes very similar to those in the human being with acute septicemia, ulcerative endocarditis, and septic phenomena. In one animal, the kidney showed lesions which were suggestive, though equivocal, of those seen in human glomerulonephritis. In another, the lesions in the heart were somewhat similar to those seen in certain collagen diseases.

STRUCTURAL ALTERATIONS WITHIN THE AORTIC INTIMA IN INFANCY AND CHILDHOOD. John T. Prior and David B. Jones (by invitation), State University of New York Medical Center at Syracuse University, Syracuse, N.Y.

Abstract. The entire length of the aorta from 50 consecutive necropsies from the pediatric service was preserved and sectioned according to the serial block technic. A variety of stains were used, including hematoxylin and eosin, Verhoeff's elastic stain, periodic acid, Gomori's silver methenamine stain, Wilder's stain for reticular fibers, Masson's trichrome stain, and buffered toluidine blue for evidence of metachromatic material. Focal intimal thickenings were noted in one of the 39 infants below 2 weeks old and in each of the remaining 11 ranging between 2 weeks and 14 years of age. These thickenings took the form of plaques which were not visible to the naked eye but could be differentiated from other intimal alterations considered to be part of normal development. The plaques were elongated in a longitudinal plane with a thickness averaging more than one-half that of the underlying media and a diameter occupying up to one-half of the vessel circumference. The thoracic and lumbar aorta were the most frequent sites of this intimal thickening. Although changes in the endothelial cells of the plaques were noted, the most striking alteration was the enormous deposition of elastic tissue in which the only cellular elements present appeared to be fibroblasts with elongated fibroglial processes. Lesser amounts of collagenous fibrillar material were noted and condensations of metachromatic

staining material were seen also. Although considerable splitting and fraying of the internal elastic membrane beneath the plaques was present, definite proof that the new elastic tissue was derived from this structure was lacking. Because the contours and the anatomical location of these plaques corresponded so closely with the lesions of adult atherosclerosis, it is suggested that these intimal thickenings may represent the earliest phase in the development of the adult lesion.

AGING PROCESSES IN THE ARTERIAL AND VENOUS SYSTEMS OF THE LOWER EXTREMITY. Morton D. Pareira (by invitation), Fred P. Handler (by invitation), and Herman T. Blumenthal, the Jewish Hospital, St. Louis City Hospital, and St. Louis University School of Medicine, St. Louis, Mo.

Abstract. Aging, as used in the discussion of the experimental data presented, refers to factors of stress and strain which, over a prolonged period, produce degenerative changes in the walls of both arteries and veins. The distribution of such degenerative changes depends in large part on hydrostatic pressures which vary as a function of size of vascular lumen and blood pressure (Law of Laplace). The progressive degenerative changes described consist of calcification, fragmentation, and fraying of elastic elements, as well as calcification of non-elastic elements of the vessel wall. Severe changes lead to bone formation in elastic elements as well as in non-elastic components, but the changes in the elastic tissue are much more frequent. So-called Mönckeberg sclerosis may consist of either or both of these types of calcification, but the type which primarily involves elastic elements is fundamentally no different from that observed in so-called elastic arteries. The relation of fragmentation and fraying of the internal elastic lamella to the formation of intimal atheroma is discussed from the standpoint of the vessel wall as an organ which carries on independent metabolic processes conditioned by physical and chemical changes; the release of lipids within the vessel wall may be a product of such processes.

THE ABSENCE OF LIPID IN PULMONARY ARTERIOLAR SCLEROSIS. David M. Spain and Judith Rosenshein (by invitation), Department of Laboratories and Research of Westchester County, Valhalla, N.Y.

Abstract. Despite many differences in anatomical alterations between systemic arteriolar sclerosis and atherosclerosis of larger vessels there is one feature in common: both frequently contain deposits of lipid. Some believe this lipid to be an important factor in development of the arteriolar sclerotic lesion. These deposits occur as frequently in the walls of renal arterioles as in the intima of larger arteries. In both, the incidence increases with age, hypertension, and diabetes. Lipid is less frequently found in hyalinized arterioles of other organs. The renal afferent arterioles are subjected to relatively high pressures which are accentuated because these arterioles arise directly from arteries several times their size. These vessels course through solid tissue that is unchanging in size or consistency. It seemed, therefore, of interest to determine the degree of lipid deposition that might occur in pulmonary arteriolar sclerosis.

In the lung the intra-arteriolar pressure is relatively low even in pulmonary hypertension; the precapillary arterioles run a short course and are derived from vessels that gradually decrease in size. The vessels course through an areolar organ that constantly changes in size and is in constant motion. The pulmonary arteriolar system, furthermore, contains blood of venous type. There are no vessels in the lung that are strictly comparable to the afferent arterioles of the kidney. Nor is the hyaline change ever as pronounced. Frozen sections of lung stained with Sudan IV were studied from 25 necropsies of patients with diffuse pulmonary arteriolar sclerosis. All small vessels were examined for lipid.

Associated vascular changes in lungs with arteriolar sclerosis may consist of proliferation of the intima, hypertrophy of the media, alterations of the elastica, adven-

titial fibrosis and/or hyalinization of the media. The larger pulmonary arteries contained plaques with lipid, but in not a single instance did the smaller arteries or arterioles contain any lipid (Sudan IV).

Two cases were of particular interest. In the first, with pulmonary hypertension secondary to silicosis, the entire vascular supply to the left lung originated from the aorta. Arteriolar sclerotic vessels in the left lung contained no lipid despite being supplied by the systemic circulation containing oxygenated blood and a considerably higher intravascular pressure. Relative to this, Benda described a case with lipid-containing sclerosis of the pulmonary veins and Wilens (personal communication) has observed a similar case with no involvement of the pulmonary arteries in a case of mitral stenosis and diabetes. However, it is claimed that no differences exist in the content of venous and arterial blood as to cholesterol and associated constituents.

The second case was a patient with pulmonary tuberculosis, emphysema, pulmonary hypertension, and complete thoracoplasty of 8 years' duration. Again lipid was not demonstrated in the arterioles despite the fact that one lung was compressed and motionless. It has been postulated that in normotensive individuals lipid is found in hyalinized arterioles in such organs as the spleen because rapid changes in volume of this organ may alter the pulp pressure so that a pressure exists favorable for the filtration of lipid from the blood into the vessel wall.

The amount of lipid found in the renal arterioles increases in proportion to the degree of hyaline sclerosis that is observed in them. Lipid is occasionally present before hyaline sclerosis has developed. This obviously is not the case in the precapillary arteriolar sclerosis that occurs in the lungs.

CEROID-LIKE PIGMENTS, HEMOCEROID AND HYALOCEROID, IN ATHEROMATOUS LESIONS OF HUMAN SUBJECTS. W. Stanley Hartroft (by invitation), Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

Abstract. The "occasional finding of 'ceroid' about focal degenerative lesions, such as atheromatous plaques" was noted by Pappenheimer and Victor (1946). Ceroid is the name given by Lillie *et al.* (1941) to the orange-brown pigment in cirrhotic livers of choline-deficient rats. This material is characterized by insolubility in alcohol, xylol, and most other fat solvents, and by intense sudanophilia in paraffin sections. It was discovered (Hartroft, 1951) that a substance, histochemically identical with ceroid, can be demonstrated both *in vitro* and *in vivo* following prolonged contact of red blood cells with fat. In this laboratory it was subsequently shown that when tissues, other than erythrocytes, are similarly treated with unsaturated fats, ceroid-like products are formed (Casselman, 1951), but their amount and the degree of sudanophilia are not as great as when red cells are used. Small hemorrhages or surface accumulations of erythrocytes in or around lipid deposits in atheromatous arteries of human subjects constitute clinical duplications of the experimental conditions described above.

It was thought on the basis of these facts that significant amounts of ceroid-like pigments might be consistently found in atheromatous lesions. This has proved to be true. In necropsy material obtained from the Department of Pathology (Toronto), pigment histochemically identical with ceroid was present, usually in abundance, in every atheromatous lesion examined in 25 aortas, in 20 of 23 coronary arteries, and in a smaller series of miscellaneous atheromatous vessels (renal, iliac, femoral, and cerebral arteries). Thrombi, unassociated with atheroma, in pulmonic arteries and in a number of veins (portal, splenic, jugular, iliac) did not contain ceroid-like products. Most of the pigment in atheromatous plaques was often closely intermingled with red cells and was probably derived in part from the latter; this type has been termed hemoceroid. Hemoceroid is characterized by an intense sudanophilia and a granular, highly refractile appearance which corresponds to the ceroid-like substance

produced *in vitro* and *in vivo* from red cells. These features distinguish it from a histochemically similar pigment, also present in the atheromatous lesions, which typically exhibits a smooth, glassy appearance with only moderate sudanophilia. This is found in hyalinized tissue around spaces which in frozen sections frequently contain stainable fat, and it has therefore been termed hyaloceroid. It resembles the product obtained in the *in vitro* experiments (Casselman) by mixing fat and tissues other than red cells. Formation of both hemoceroid and hyaloceroid may prove to be an important factor in the explanation, at least in part, of the progressive nature and apparent irreversibility of atheroma.

EXPERIMENTAL ATHEROSCLEROSIS IN THE RAT. Joseph H. Bragdon (by invitation) and Edwin Boyle (by invitation), National Institutes of Health, Bethesda, Md.

Abstract. Hitherto the rat has remained resistant to all attempts at the production of experimental atherosclerosis. When the low-density lipoproteins from the serum of cholesterol-fed rabbits are injected intravenously in rats, atherosclerotic-like lesions appear in the recipients. The lipoproteins are isolated and concentrated by centrifugal flotation at 30,000 r.p.m. after increasing the saline density of the serum to a sp. g. of 1.063. The excess salt is then removed by dialysis. Within 24 hours following a single injection of this material, sudanophilic granules may be seen within occasional endothelial cells of the larger arteries. Following repeated daily injections, focal lesions appear which are characterized by lipid-swollen endothelium and scattered lipid granules in the underlying media.

THE RÔLE OF ARTERIAL INJURY IN THE LOCALIZATION OF METHYL CELLULOSE. L. L. Waters and R. S. Duff (by invitation), Yale University School of Medicine, New Haven, Conn.

Abstract. Intravenous injection of high viscosity methyl cellulose into dogs is well known to result in foam-cellular lesions of the aorta and coronary arteries. It was found that 1 cc. per kg. of a 2 per cent aqueous solution given on alternate days over a period of 2 weeks resulted in only an occasional microscopic lesion in the aorta and not any changes in the coronary arteries. When, however, the coronary arteries of dogs had been injured by the injection of allylamine, subsequent administration of high viscosity methyl cellulose, as outlined, was followed by massive foam-cellular changes in the coronary arteries selectively at the sites of allylamine injury. These foam-cellular arterial lesions often occluded medium-sized coronary vessels in a period of 2 weeks. In dogs given allylamine followed by medium viscosity methyl cellulose, similar lesions of the coronary arteries occurred accompanied by deposits of stainable fat in the intimal lesions.

These experiments demonstrate that at this level of dosage the localization of methyl cellulose is dependent upon prior injury of the arterial wall. The lesions produced by combining allylamine and methyl cellulose injections are demonstrated and their significance discussed briefly.

PATHOGENESIS OF POLYARTERITIS NODOSA IN HYPERTENSIVE RATS. George J. Race (by invitation) and Ernst Peschel (by invitation), Duke University Hospital, Durham, N.C.

Abstract. During the course of investigations of the effects of dietary factors upon young hypertensive rats, a characteristic panarteritis similar to human polyarteritis nodosa frequently developed in those animals which received varying amounts of supplementary sodium chloride. The animals used were 20 normal and 80 hypertensive females of the Osborn-Mendel strain which were made hypertensive by renal encapsulation and subsequent contralateral nephrectomy. The rats developed initially a characteristic sustained hypertension of 180 to 220 mm. of Hg, often followed by rises up to 300 mm. of Hg or more. Diets used consisted of varying mixtures of

carbohydrates and proteins given in the natural food stuff with varying amounts of sodium chloride. The hypertensive rats died at an average age of 127 days. Complete necropsies revealed an incidence of polyarteritis nodosa of 31.2 per cent; involved in order of decreasing frequency were arteries of mesentery, pancreas, spleen, extremities, ovary, and heart. No incidence of polyarteritis nodosa occurred in the normal animals. Grossly the lesions appeared as tortuous adherent nodules ranging from 1 to 10 mm. in diameter which involved the medium-sized arteries. Microscopically, the arterial changes, studied with multiple special staining technics, were invariably those of panarteritis. Three stages of development based on necrosis, presence and type of inflammatory exudate, and degree of healing were observed: an acute, a subacute, and a healing stage. The acute stage was characterized by a fibrinoid necrosis of the intima and inner media with acute inflammatory exudation and edema of the outer media and adventitia. The subacute stage showed mixed inflammatory infiltration and early evidences of healing. Often thrombosis was observed. The healing stage was characterized by massive fibrosis with complete disorganization of the vessel wall. Recanalization of thrombi occurred and calcification of the intima and media was common. These observations are contrasted with lesions of similar nature previously described in the literature. The differences and agreements are discussed in the paper.

MORPHOLOGIC ALTERATIONS IN THE KIDNEY DURING PROLONGED EXPERIMENTAL RENAL AND NEUROGENIC HYPERTENSION IN THE DOG. Melvin L. Goldman (by invitation), Henry A. Schroeder (by invitation), and Gustave J. Dammin, Washington University Medical School, St. Louis, Mo.

Abstract. This study is concerned with the relationship of prolonged experimental hypertension in the dog to morphologic alterations in the kidney, as demonstrated by periodic biopsy. Three groups of dogs, group I (3 dogs) with hypertension induced by moderator nerve resection, group II (5 dogs) in which the Goldblatt renal artery clamp or silk pouch was applied, and group III (2 dogs) in which both moderator nerve resection and the Goldblatt or silk pouch procedure were used, have been observed for a period of over 4 years. Approximately 50 dogs were observed over a shorter period of time.

In group I, the sequence of study consisted of (a) a control period of blood pressure observation, (b) biopsy of one kidney, (c) moderator nerve resection, and (d) periodic biopsy of the contralateral kidney. In group II, there was (a) a control period of blood pressure study, (b) biopsy of one kidney and application of a Goldblatt clamp or a silk pouch on the same side, and (c) periodic biopsy of the contralateral kidney. In group III, the (a) control period was followed by (b) biopsy and application of the Goldblatt clamp or silk pouch to the same kidney, then (c) moderator nerve resection and (d) periodic biopsy of the contralateral kidney. The Heidenhain azocarmine-aniline blue, the periodic acid leukofuchsin, and hematoxylin and eosin stains were performed on all biopsy specimens; Masson's trichrome stain was used occasionally in addition.

In some degree all of the dogs in groups I and II and one of the 2 dogs in group III showed the following morphologic alterations, in decreasing order of prominence: (1) a thickening of the basement membrane of the glomerular capsule; (2) a thickening of the basement membrane in the glomerular tuft, and (3) an increase in intercapillary substance. These changes were best demonstrated by the periodic acid leukofuchsin staining method. They usually appeared between the 6th and 12th month after the operative procedure and progressed slowly. No alteration in thickness of the arteriolar walls, as demonstrated by measurement, could be demonstrated, nor was there any qualitative change noted in the arteriolar walls. There was no wrinkling of the basement membrane of the glomerular tuft, as described in essential hypertension. No alteration was observed in the juxtaglomerular complex or in the

basement membrane of the tubules. The absence of arteriolar alteration in prolonged experimental renal and neurogenic hypertension, as noted in this study, coincides with the observations of Goldblatt. The thickening of the basement membrane of the glomerular capsule and that of the glomerular tuft and the increase in intercapillary substance, changes listed in order of decreasing prominence, are reported as morphologic alterations occurring in most dogs in which neurogenic or renal hypertension has been induced and maintained for long periods.

THE EFFECTS OF FLUID AND ELECTROLYTE BALANCE ON BILATERALLY NEPHRECTOMIZED DOGS. J. L. Orbison and (by invitation) C. W. Christian and Evelyn Peters, Western Reserve University, Cleveland, Ohio.

Abstract. Three groups of bilaterally nephrectomized dogs were studied: (1) dogs given no food or fluid after the operation; (2) dogs given 100 cc. of 0.85 per cent NaCl per kg. per day intraperitoneally; (3) dogs given 100 cc. of balanced salt solution per kg. per day intraperitoneally. On all of these animals determinations were made to identify any changes in arterial blood pressure, serum Na, K, Ca, and Cl, blood volume, inulin space, and permeability of the skin to India ink and T 1824 and also to identify the occurrence of acute arterial necrosis.

The dogs given sodium chloride solution and those given a balanced salt solution had a pronounced increase in blood pressure within 24 to 48 hours, whereas those given no fluid had no significant increase throughout the 3 to 5 days which they lived after bilateral nephrectomy. Similarly the 2 groups given the salt solutions had a much greater degree of vascular necrosis than the group given no fluid. Since no significant difference in blood volume was found among the groups, the elevated blood pressure and arterial necrosis are not correlated with blood volume. They do correlate, however, with the increase in inulin space and skin permeability as demonstrated in both groups given salt solutions. Electrolyte determinations are not yet complete.

STUDIES ON THE PATHOGENESIS OF GLOMERULONEPHRITIS. Howard C. Hopps and (by invitation) Florence C. Kelly and Raymond L. Engles, University of Oklahoma School of Medicine, Oklahoma City, Okla.

Abstract. *Streptococcus pyogenes*, NY5 strain, was cultured on non-antigenic medium (Bernheimer). The filtrate (Dick toxin) had a potency of approximately 300,000 STD per ml. This material (0.1 ml.) was injected into rabbits four times weekly for 33 weeks, followed by a 16-week "rest period." After this, injections were resumed for 11 weeks. Animals were necropsied and histopathologic studies made, some at the end of this period, others during the course of treatment.

CHRONIC GLOMERULONEPHRITIS AND NEPHROTIC SYNDROME INDUCED IN RATS BY N,N′-DIACETYL-BENZIDINE. John W. Harman and (by invitation) Elizabeth C. Miller and James A. Miller, University of Wisconsin Medical School, Madison, Wis.

Abstract. Groups of Sprague-Dawley rats, of both sexes and the same initial weight, were fed a grain diet containing 0.043 per cent N,N′-diacetyl-benzidine. Within 3 to 4 weeks a considerable proteinuria was observed in both sexes. The proteinuria rapidly became severe in the females, until within 2 months as much as 0.1 gm. of protein was excreted within 24 hours. The females were edematous and severely anemic by about the 10th to 12th week on the diet, with some weight loss and weakness. Blood counts established red cell levels of 900,000 to 2,000,000 per cmm. (8,000,000, normal) and frequent polymorphonuclear leukocytosis. Marrow studies revealed hypoplasia with retarded erythropoiesis. There were both hypoproteinemia and lipemia.

Necropsies were performed on all rats when they attained a degree of anasarca and

asthenia suggesting impending death. In all instances the subcutaneous edema, ascites, anemia, and severe lipemia were confirmed in the females. The kidneys were regularly small, brown, and granular, and had poor corticomedullary differentiation. No other gross lesions were seen regularly in females. Microscopically, there was a proliferative glomerulonephritis affecting nearly all glomeruli, with epithelial hyperplasia and crescent formation. Fibrosis was seen occasionally but was of slight degree. Many tubules were plugged with hyaline and eosinophilic casts. In several instances bacterial endocarditis was encountered on mitral leaflets.

In contrast with the females, the male rats developed proteinuria more gradually and manifested only a mild nephrotic syndrome after 5 months. The renal lesions were less extensive. In addition, however, there was atrophy of the testes in all males which showed the syndrome. Neither sex developed the disease on the diet devoid of the N,N′-diacetyl-benzidine.

THE EFFECT OF INORGANIC URINE PHOSPHATE ON THE DEVELOPMENT OF HEMOGLOBINURIC NEPHROSIS IN RABBITS. Joseph J. Lalich, University of Wisconsin Medical School, Madison, Wis.

Abstract. Experimental evidence suggests that related factors such as antecedent tubular injury, depletion of extracellular fluid, and injections of histamine or arsine are more influential than hemoglobinemia in the production of hemoglobinuric nephrosis. Since inorganic phosphate may be lost from injured muscle and phosphate is nephrotoxic, it seemed advisable to study its influence on the production of hemoglobinuric nephrosis. The plasma concentration and urine excretion of inorganic phosphate were studied in 5 control and 23 rabbits which received 1 gm. per kg. of oxyhemoglobin or methemoglobin. Urine phosphate excretion was increased by supplementing ground oats with mixtures of mono- and dibasic sodium phosphate salts. Intraperitoneal injection of 70 mg. per kg. of phosphorus as buffered (pH 7.0) 1 per cent sodium phosphate was combined with 3 days of starvation in 5 control rabbits. This produced eosinophilic casts and foci of lymphocytic infiltration in the kidneys of 4 of 5 rabbits. Injections of acidified and neutralized oxyhemoglobin into 8 rabbits which were fed a 1 per cent mixture of sodium phosphate in ground oats failed to produce significant numbers of pigment casts in the distal convoluted tubules. In 4 animals given oxyhemoglobin and 11 others in which injections of oxyhemoglobin were combined with intraperitoneal administration of buffered 1 per cent sodium nitrite or potassium ferricyanide, phosphate feeding did not appear to increase the pigment cast accumulation in the kidneys. The methemoglobin-producing salts predisposed to pigment cast formation independently of the urine phosphate concentration. In contrast to the results observed following starvation and intraperitoneal injection of sodium phosphate, when the salt was fed in ground oats it was not nephrotoxic. It is concluded that phosphate may exert a nephrotoxic effect in rabbits when the plasma phosphorus concentration is sustained for 2 or more days at concentrations in excess of 8 mg. per cent.

THE STRUCTURAL BASIS OF SICKLING AND ITS ELECTRON MICROSCOPY. John W. Rebuck and (by invitation) R. W. Monto and R. M. Sturrock, Henry Ford Hospital, Detroit, Mich.

Abstract. The structural changes which occurred during the sickling process were followed in electron micrographs of erythrocytes obtained from 5 patients with sickle cell trait and 3 patients with sickle-cell anemia. By a modified Beck-Hertz technic, formalin fixation at successive time intervals arrested the sickling process at progressive stages in the transformation of the normal-appearing promeniscocyte to the sickled cell. Early, there was an irregular aggregation of hemoglobin at one side of the cell. Focal thinning of the corpuscular center was sometimes accompanied

by anisotropoid angulation of central aggregates of hemoglobin. After 5 hours of anoxia, the eccentric, hemoglobin-containing intracorpuscular masses presented intense peripheral spiculation suggestive of incipient crystallization. Trait cells presented less angulation of their hemoglobin aggregates than did their anemia counterparts. Confirmation of Pauling's concept that the defective reduced hemoglobin of the sickle cell is capable of entering into three dimensional patterns was presented in micrographs of shadow-cast sickle cells with projection of linear and other aggregates to varying heights. The *cellular* confirmation of Harris' hemoglobin tactoids was shown as well as corpuscular configurations transitional between tactoids and classical sickled forms. This spindle-shaped orientation of hemoglobin-containing aggregates within the cell has been compromised both by forces inherent within still intact portions of the surface ultrastructure and by membranous filamentation. The concept of physiologic accelerators of sickling introduced by Sydenstricker has been reinvestigated from the structural standpoint. Soap and bile salts in small concentration were each observed to accelerate and accentuate the sickling phenomenon through lessening of the intermolecular forces in the surface ultrastructure. As a result almost free play was permitted to the opposing forces of hemoglobin orientation potentials tending to intracorpuscular crystal formation. Electron micrographs depicted each stage of the sickling process under discussion.

THE APPLICATION OF X-RAY DIFFRACTION IN PATHOLOGY (WITH PARTICULAR REFERENCE TO PULMONARY GRAPHITOSIS). H. Edward MacMahon, Tufts College Medical School and Massachusetts Institute of Technology, Boston, Mass.

Abstract. Our problem was to identify a black crystalline pigment present in large quantities throughout the lung of an electrotype molder who had been exposed to graphite dust for years. Necropsy showed a very severe degree of pneumoconiosis, accompanied by dilatation and hypertrophy of the right ventricle. No silica was demonstrable by polarization in our tissue sections or in the graphite dust used by this patient. Since carbon black is generally considered to be relatively innocuous to pulmonary tissues, it seemed to us important to attempt to identify the black crystalline pigment deposited in all areas of pulmonary fibrosis. To do this, samples of graphite used by this patient were carefully studied, measured, and compared with the crystalline material in both the lung and regional lymph nodes of this patient. Using an ordinary microscope, the similarity of the two was striking. With a Leitz Ultropak microscope, the crystalline detail was so alike in the lung and in the free dust that it seemed reasonable to consider the two as the same, but it was only after submitting both of these samples to x-ray diffraction that the nature of these substances could be identified with any degree of certainty. X-ray diffraction here refers to the passage of a monochromatic beam of x-rays through a thin film of finely ground crystalline powder. A series of concentric cones representing diffracted rays emerges from this powder. When a photographic plate is placed at right angles to the initial beam passing through the powder, a series of concentric circles will appear on the developed plate. These represent the points at which these cones intersect the plate. The density of each circle is determined by the density of the atomic planes which give rise to the lines of diffraction. The spacing of the lines is determined by the separation of these planes. In this way a "powder diagram" may be obtained for identification of a crystalline substance. When obtained from an unknown crystalline substance, the unknown diagram can be compared with x-ray powder diagrams of known materials. Where patterns match, the powders are considered to be identical, and at the present time thousands of crystalline substances have been studied by this method.

On the basis of diffraction studies it could be shown that the lung and lymph node contained a carbonaceous material, that the carbon present in the tissues was pre-

dominantly present as graphite, and that carbon black and other undetermined crystalline impurities contaminated the graphite to which this patient was exposed. This does not answer the question whether graphite can cause pneumoconiosis; it does, however, offer us a means of studying and identifying an unknown crystalline substance such as graphite when associated with advanced pulmonary fibrosis.

HISTOCHEMICAL AND FLUORESCENT MICROSCOPIC STUDIES OF GROUND SUBSTANCE. Walter Joel (by invitation) and Howard C. Hopps, University of Oklahoma Medical School, Oklahoma City, Okla.

Abstract. Histochemical and fluorescent microscopic methods were applied to the study of ground substance in normal tissue and tissues altered by a variety of allergic inflammatory and related diseases. In some instances these tissues were prepared by the freezing-drying technic. These findings were presented with discussion of their essential nature and importance.

LOSS OF TUMOR-IMMUNE ACTIVITY FROM SERUM FOLLOWING EXPOSURE TO INJURED LYMPHOID CELLS. Herbert C. Stoerk, Merck Institute for Therapeutic Research, Rahway, N.J.

Abstract. Previously it was observed that sera of rats, in which lymphosarcoma implants had regressed, suppressed the growth of lymphosarcoma cells in susceptible animals of the same stock (Sprague-Dawley). Such homologous immune sera, unlike heterologous immune sera and unlike the homologous immune sera of one highly inbred strain of mice against tumors of another strain (Gorer), did not measurably react *in vitro* with rat blood cells or even with the tumor cells. It therefore became apparent that a factor supplied by the susceptible animal was essential for this immune reaction. Since this factor was not contributed by fresh guinea-pig or rat serum, it does not appear to be complement. Also, in accordance with Kidd's and Toolan's findings, lymphosarcoma cells mixed with a suspension of *intact* lymphoid cells from rats resistant to reinoculation with lymphosarcoma failed to grow in susceptible rats. Since exposure to moderate heat (40° C. for 15 minutes) or freezing abolished this activity, it appeared dependent upon the integrity of the lymphoid cell. Several other biologic activities of lymphoid cells likewise have appeared to be dependent upon the intact state of these cells: passive transfer of "delayed" hypersensitivity (Landsteiner and Chase), protection from injury following x-radiation (Jacobsen), resistance to tumor grafting, and production of splenic enlargement with homologous lymphoid cells (Stoerk), all could be achieved with intact—but not with injured—lymphoid cells. These findings have usually been interpreted as indicating that certain biologic actions of lymphoid cells are dependent upon the activities of the live cell. However, they may also be assumed to mean that in intact cells active principles, by spatial separation, may be protected from "inactivators." Injury to the cell with destruction of a protective barrier may render "inactivators" effective.

Evidence to support this possibility was obtained from experiments showing that tumor-immune activity of rat serum was undiminished following contact with suspensions of intact lymphoid cells, but was lost during exposure to identical suspensions after freezing or heating (40° C. for 15 minutes). Loss of immune activity was observed with autologous, homologous, and heterologous cells alike. On the assumption that the inability to extract immune activity from "immune" lymphoid cells by freezing and thawing was due to the action of an "inactivator," and that this inactivation would not occur instantaneously, attempts were made to separate the immune principle from its "inactivator." This was met by success in about one-third of the cases in which suspensions of "immune" lymphoid cells were precipitated with 15 per cent $NaSO_4$. The gamma globulin fraction so obtained possessed immune activity.

THE DETERMINATION OF THE MASS OF HISTOLOGIC STRUCTURES BY X-RAY ABSORPTION. Patrick J. Fitzgerald and (by invitation) H. G. Beyer, N. T. Williams, and H. Sherman, Sloan-Kettering Institute, Memorial Hospital Center, New York, N.Y.

Abstract. The technic of Engstrom has been used to determine the relative mass of various histologic structures in human and animal tissues. Absolute mass determinations of small biologic units at the cell level are possible and this makes feasible the determination of the dry weight of some cells and cell units. The apparatus and technic were illustrated briefly and representative microradiographs of human and animal tissues demonstrated.

PRIMARY ALVEOLAR CELL CARCINOMA OF THE LUNG. John H. Fisher, University of Western Ontario, London, Canada.

Abstract. Five cases of so-called primary alveolar cell tumor of the lung are reported. All were malignant, producing regional or distant metastases. In all cases both lungs were involved. In 2, the tumor was multiply nodular in type while in 3, it was of a combined nodular and diffuse form. The histologic structure of the two anatomical forms is identical. In structure and biologic behavior the tumors resembled a carcinoma. For reasons presented, the opinion is expressed that these tumors arise from terminal bronchiolar epithelium. Others have called them "cancerous pulmonary adenomatosis." No case of benign pulmonary adenomatosis has been encountered. In all instances the tumor fulfilled the criteria set forth by Swan. The gross and microscopic features correspond closely to those described by Swan and by Neubuerger and Geever. The combination of its unique histologic structure, its gross characteristics unusual for other pulmonary neoplasms, and its more or less constant roentgenologic picture, serve to place this tumor in a separate category. No constant causal factor has been encountered in the clinical histories. The ratio of males to females was 3 to 2. Ages ranged from 47 to 80. In all 5 cases the clinical signs and symptoms were those of chronic pulmonary disease. It is considered that the widespread tumor growth in the lungs interfered significantly with normal pulmonary function and thus was an important contributory cause of death.

Since submitting the title of this paper, a sixth case has been encountered in a man, 47 years old, with a scattered, finely mottled infiltration of both lung fields and with invasion of the 5th lumbar vertebra and right supraclavicular lymph nodes. Biopsy of the vertebra and a lymph node showed a characteristic histologic appearance.

TRANSPULMONARY PASSAGE OF TUMOR CELL EMBOLI. Irving Zeidman and JoAnne M. Buss (by invitation), University of Pennsylvania Medical School, Philadelphia, Pa.

Abstract. Experiments were performed to settle the much debated and recently emphasized controversy concerning the possibility of immediate passage of cancer emboli through the lung circulation. The currently accepted opinion is that un-arrested transpulmonary passage occurs rarely, if at all; metastases to such systemic organs as kidneys and adrenal glands are believed to result from tumor emboli released by secondary pulmonary growths. Experiments were designed to test this concept, using V_2 carcinoma and Brown-Pearce carcinoma in rabbits, and Walker carcinoma 256 in rats. A suspension of tumor cells was injected intravenously and simultaneously the aortic blood was collected in a syringe. This aortic blood was then injected intravenously into a second normal animal, and the animal was sacrificed 1 month later. The presence of tumors in the second animal would indicate immediate transpulmonary passage of cancer cells in the first animal. It was found that each cancer used passed through the lungs immediately in some experiments,

and the highest incidence of passage occurred with the Brown-Pearce carcinoma. It is concluded that immediate transpulmonary passage of tumor cell emboli occurs readily, and it is suggested that this passage may be a frequent event rather than a rare one, as previously considered.

THE SIMILARITY OF THE PREGNANCY EFFECT ON THE EPITHELIUM OF THE MATERNAL AND FETAL CERVIX. Louis M. Hellman and A. H. Rosenthal (by invitation), Kings County Hospital, Brooklyn, N.Y.

Abstract. Pregnancy exerts a profound effect on the maternal cervix. There is hyperplasia of the glands and of the epithelium lining the glands, with formation of new glands. In the ectocervix there is basal cell hyperactivity and occasionally there occur changes similar to intraepithelial carcinoma which are, however, reversible. In the fetus the same changes occur in the glands to a very marked degree. They seem to be related to the "reserve cell." In addition, basal cell hyperactivity of the ectocervical epithelium occurs. These changes may be related to estrogen stimulation.

PROBABLE HODGKIN'S DISEASE IN A DOG. Frank Bloom, State University Medical Center of New York, Brooklyn, N.Y.

Abstract. Authentic descriptions of spontaneous lesions in animals resembling human Hodgkin's disease are of great rarity. Two probable cases have been described in dogs. A third probable case was encountered when a 7-year-old female mongrel was presented with symptoms of thirst, vomiting, diarrhea, dullness, loss of weight, and a palpable mass in the abdominal cavity. The essential gross lesions were as follows: The mesenteric lymph nodes were nodular, greatly enlarged, firm, gray to gray-yellow, and measured 9 by 4 cm.; the mesentery, small intestines, and pancreas were adherent to the nodes. The terminal portion of the small intestine was involved in an ovoid thickening 7.5 cm. long and 3.2 cm. wide; the intestinal lumen in this region was narrowed and measured 0.6 cm. in diameter. On section, all coats were diffusely replaced by tissue similar to that of the mesenteric nodes. The spleen was slightly enlarged, dark, and contained several gray, subcapsular, elevated nodules varying from 1 to 3.5 cm. in diameter. The liver was enlarged and contained innumerable minute gray foci. The right perirenal lymph node was enlarged, measured 1.5 by 2.7 cm. and had the same appearance as the mesenteric nodes. The anterior mediastinal lymph nodes also resembled the mesenteric nodes and formed a large conglomerate nodular mass, 3.6 by 5.8 cm., which was attached to the chest wall in the left costosternal region. All other tissues and organs appeared normal.

With few exceptions, the microscopic appearances were alike and were limited to those organs showing gross lesions. The normal lymph node architecture was replaced completely by intermingling of dense masses of large pale reticulo-endothelial cells, diffuse eosinophils, lymphocytes, plasma cells, polymorphonuclear leukocytes, and histiocytes often containing cytoplasmic pigment. Only rarely were giant cells of the Sternberg-Reed type found. In some regions the structure consisted mainly of reticulo-endothelial elements and resembled reticulum cell sarcoma. Other areas were composed mainly of eosinophils. Fibrosis was not prominent but here and there the connective tissue was increased. The capsule and perinodal fat contained similar cellular infiltrations which invaded the adjacent muscle of the thoracic wall and the mesenteric and peripancreatic fat. Small and large areas of necrosis were distributed irregularly. The same pleomorphism and distribution of cell types were observed in the thickened portion of the small intestine and all coats showed replacement of the structure by diffuse infiltrations. The gray nodules in the spleen resembled the involved lymph nodes and were not sharply demarcated. The liver contained numerous round, oval or irregular foci irregularly distributed throughout the parenchyma and beneath the capsule. The cellular types in these foci were the same as in the

other organs except for greater numbers of eosinophils.

With the exception of the sparse Sternberg-Reed cells, the lesions appear to fulfil the morphologic criteria for Hodgkin's disease in man. As such, the condition in the dog is classified as probable Hodgkin's disease.

RETICULUM PATTERN IN SMOOTH MUSCLE TUMORS. R. M. Mulligan, University of Colorado, School of Medicine, Denver, Colo.

Abstract. The value of staining reticulum fibers in the differentiation of neoplasms of fibroblasts and of nerve sheath cells has long been recognized. Although trichrome stains have been found useful in the detection of myofibrils in smooth muscle tumors, the reticulum pattern of these tumors has received relatively little attention. In order to determine whether reticulum impregnation might be of value in the differential diagnosis of neoplasms of smooth muscle, 11 leiomyomas (10 uterine and one cutaneous in women 28 to 78 years old) and 5 leiomyosarcomas (4 uterine and one retroperitoneal in women 30 to 71 years old) were stained with hematoxylin and eosin and with Wilder's reticulum stain counterstained with van Gieson's method. A fibroma of the finger, a fibrosarcoma of the neck, a diffuse neurilemmoma (neurofibroma) of the sacral region, a solitary neurilemmoma of the mediastinum, and a malignant neurilemmoma of the thigh were used as controls. Conclusions concerning the character of reticulum fibers were drawn from study of well preserved areas of each tumor where the neoplastic cells were cut longitudinally. In both leiomyomas and leiomyosarcomas the caliber of the reticulum fibers ranged from fine to coarse. The fibers were disposed between cells, were often marked by short to medium, fairly evenly distributed waves, and numbered 1 to 3 in tandem in a × 450 field. The fibers commonly displayed both terminal and lateral splitting. The most conspicuous characteristic was the frequent formation of fine lateral branches either passing among tumor cells or wrapped circumferentially about them. Terminal and lateral splitting was observed frequently only in the diffuse neurilemmoma. Lateral branching was found in none of the control tumors.

INFLAMMATORY FIBROID POLYPS OF THE STOMACH. Elson B. Helwig and Andrew Ranier (by invitation), Armed Forces Institute of Pathology, Washington, D.C.

Abstract. The polypoid lesions of the stomach observed at the Armed Forces Institute of Pathology include 9 examples of a solitary polyp comprised chiefly of fibrous tissue and hyperplastic blood vessels accompanied by inflammatory infiltration. The fibrous tissue was generally disposed as delicate fibrils and in the vicinity of blood vessels often assumed a concentric onion-skin-like pattern. The inflammatory infiltrate was composed predominantly of eosinophilic leukocytes but also included plasma cells and scattered lymphocytes. Mucosal glands covering the polyp showed simplification and were separated by a loose stroma variously infiltrated with plasma cells, eosinophils, and lymphocytes. The polyp extended from the submucosa toward the lumen and commonly interrupted the muscularis mucosae. The overlying mucosa occasionally was ulcerated. In 8 of 9 polyps the location was specified as the pyloric region. The polyps were sessile or pedunculated and ranged from 1 to 5 cm. in diameter. Seven of the 9 patients complained of gastric symptoms including epigastric and abdominal pain. Clinical studies of 3 patients disclosed pernicious anemia and of one, achlorhydria. Two of the lesions were incidental observations at necropsy. The ages of the patients ranged from 22 to 90 with a median age of 58.

Similar lesions have been recorded under such diverse terms as eosinophilic granuloma of the stomach, gastric submucosal granuloma with eosinophilic infiltration, hemangio-endothelioma, hemangiopericytoma, neurofibroma, and atypical schwannoma. On the basis of the present observations it is postulated that the lesion represents a reaction of the gastric mucosa and submucosa to some form of inflammatory stimulus. Chronic gastritis as determined by examination of the contiguous gastric

mucosa was present in all of the lesions. The association of pernicious anemia in 3 patients appears to be more than coincidental.

UNTREATED BRONCHOGENIC CARCINOMA: A CLINICOPATHOLOGIC STUDY OF THIRTY-FIVE CASES AND REVIEW OF THE RECENT LITERATURE. George R. Tanner (by invitation) and Harold Gordon, Veterans Administration Hospital, and University of Louisville School of Medicine, Louisville, Ky.

Abstract. Data have been assembled from the case records of 35 patients who died of untreated bronchogenic carcinoma. These were collected from a series of 900 consecutive necropsies performed between April, 1946, and December 31, 1951, at the Veterans Administration Hospital, Louisville, Ky. They can be divided into five groups, according to the type of cancer:

Epidermoid	18
Anaplastic	13
Mixed (epidermoid adenocarcinoma)	2
Adenocarcinoma	1
Terminal bronchiolar	1

There was no significant difference in the site of origin of the epidermoid and anaplastic carcinomas, both types tending to arise from the main stem or the lobe bronchi. The epidermoid carcinomas tended to be larger and terminated at a later average age than the anaplastic. Many of the epidermoid carcinomas metastasized to distant organs. This is attributed to inhibition of infection by antibiotics, with prolongation of life. This permits the cancer to progress according to the "natural biology" of its component cells, with dissemination of tumor emboli. The brain, adrenal glands, pancreas, and liver were involved more frequently by metastases from anaplastic than from epidermoid carcinoma. For the pancreas, adrenal glands, and liver, this may be due to the tendency of the "oat cell" tumors to spread along the adventitia of the aorta. The duration of symptoms was longer in the epidermoid than in the anaplastic carcinomas. But the disease is too insidious to permit of dating its onset accurately. The symptoms, physical changes and roentgenograms were not sufficiently distinctive to permit of early diagnosis.

The cases of mixed carcinoma, adenocarcinoma, and terminal bronchiolar carcinoma were too few in this series to permit generalizations. However, our one case of terminal bronchiolar carcinoma metastasized to the peribronchial lymph nodes. We believe that the tumor started multicentrically, possibly also bilaterally.

TESTICULAR TUMORS IN INFANTS. F. K. Mostofi, Armed Forces Institute of Pathology, Washington, D.C.

Abstract. In an attempt to understand the biology and histogenesis of adult testicular and other teratomatous tumors, 25 infantile testicular tumors from the files of the Armed Forces Institute of Pathology have been studied. In this group three distinct histologic patterns were identified. There was evidence to suggest that two of these are closely related. Although over 35 per cent of adult testicular tumors are seminomas, not a single seminoma has been seen in the infantile group. The significance of this observation and its bearing on the histogenesis of testicular tumors in general was discussed.

MIXED TUMORS OF THE SKIN. Robert P. Morehead, Bowman Gray School of Medicine, Winston-Salem, N.C.

Abstract. This study was based upon a large group of mixed tumors of the skin of the head, neck, trunk, and extremities. In most instances the neoplasms studied were identical in structure with mixed tumors ordinarily encountered in the salivary glands. Occasionally tumors were encountered which were similar to the rarer types of salivary gland neoplasms. Evidence is presented which supports the contention that these tumors arise from abnormal epithelial cells which are situated in both the

epidermis and dermal appendages. These cells apparently possess developmental potentialities similar to those from which salivary gland neoplasms arise.

PHASE MICROSCOPE STUDIES OF RENAL GLOMERULI. GLOMERULAR DEPOSITS OF "HYALINE" SUBSTANCES. J. Churg and (by invitation) E. Grishman, Mt. Sinai Hospital, New York, N.Y.

Abstract. Phase microscopy lends itself readily to the study of renal glomerular architecture in fixed, paraffin-embedded material. With the aid of mounting media of high refractive index this technic allows simultaneous visualization of various structures and demonstrates particularly well the fibrillar and membranous components, such as capillary basement membranes and reticulum fibers. By combining phase microscopy with various special stains applied to serial sections, fine details of normal histology, as well as location and "inner" structure of abnormal deposits can be particularly well shown. This procedure was applied to the study of normal glomeruli and of "hyaline" deposits in amyloidosis, intercapillary glomerulosclerosis, arteriosclerosis, and disseminated lupus erythematosus. A total of 51 cases was examined.

The "hyaline" deposits differ in their inner structure as well as in their location in relation to the basement membrane of the capillary loops. In arteriosclerosis, these deposits appear in the lumen. They are of homogeneous structure and only rarely show small vacuoles. In lupus erythematosus, the deposits, so-called wire loops, lie in the wall, between the endothelium and the basement membrane. They are also homogeneous and highly refractile. In intercapillary glomerulosclerosis and in amyloidosis the "hyaline" material lies between the capillary loops, presumably in the intercapillary space. It appears non-homogeneous, spongy, or finely vacuolated. The typical Kimmelstiel-Wilson nodules have a laminated structure. The distribution of various "hyalins" and their relation to the capillary wall and the vascular root of the glomerulus are in accordance with the concept of the intercapillary space, as advocated by Zimmermann, Mollendorf, Bensley, and McManus.

THE NORMAL GLOMERULUS AND ITS BASIC REACTIONS IN DISEASE. James F. Rinehart and (by invitation) Marilyn Farquahar, Haw Chan Jung, and S. K. Abul-Haj, University of California Medical School, San Francisco, Calif.

Abstract. The normal glomerulus has been studied using histochemical methods and electron microscopy. The histochemical methods include combination staining for acid mucopolysaccharides and application of the Schiff reaction. The electron micrographs were prepared from perfused kidney of the rat by methods essentially as described by Dalton. Use of these methods has revealed structural features not previously described. The basement membrane of the glomerulus appears to have two components, a cytoplasmic extension of the endothelial cell and a substance probably of mucoid character derived from the surface epithelial cells. Thus the membrane does not appear to be truly homogeneous. Evidence is presented that it is, in a sense, porous, with a structure recalling evenly spaced rounded tiling with intervening cement lines which are probably of a mucoid nature. Evidences for this are derived from the several methods of study. The basic glomerular changes seen in glomerulonephritis, lupus, eclampsia, diabetic "glomerulosclerosis," and essential hypertension as revealed by histochemical preparations were described and illustrated.

HISTOCHEMICAL CORRELATIONS IN ISCHEMIC OBSOLESCENCE OF GLOMERULI. J. F. A. McManus and C. H. Lupton, Jr. (by invitation), University of Virginia School of Medicine, Charlottesville, Va.

Abstract. Early stages of ischemic alterations of glomeruli have been described by McGregor from studies using the Mallory-Heidenhain and Lee-Brown stains. The morphologic sequences to complete obsolescence in the ischemic glomerulus have been detailed by McManus using the PAS stain for 1,2 glycols including carbo-

hydrates. The process begins with wrinkling of the basement membrane of the capillary loops followed by the deposit of a hyaline material, strongly positive by the PAS method, internal to the capillary basement membrane. Later, another hyaline material, less positive by the PAS method, fills in the space of Bowman's capsule. The present communication reports the appearance of fibers which stain with Weigert's fuchselin and Unna's acid orcein methods for elastica, in the area of the parietal layer of Bowman's capsule during the late stages of glomerular obsolescence due to ischemia. McManus, Lupton, and Harden have reported 5-nucleotidase appearing in glomeruli *de novo* during obsolescence and McManus and Lupton have described these features in detail. ATPase is present also.

A correlation between the histologic and chemical changes in ischemic obsolescence of glomeruli is presented, utilizing the data from routine histologic methods including elastica stains and from a variety of histochemical technics including the PAS and Ritter and Oleson methods and the demonstration of phosphatase and nucleotidase enzymes. Fiber formation shown with the elastica stains is important in this dynamic process.

THE CLINICOPATHOLOGIC SIGNIFICANCE OF MULTINUCLEATED GIANT CELLS IN THE TUBULAR EPITHELIUM OF THE KIDNEY. Peter P. Ladewig, Laird Memorial Hospital, Montgomery, West Va.

Abstract. A sample group of 100 necropsy cases was studied to clarify the relationship between clinical data and the numerical and quantitative incidence of multinucleated epithelial giant cells in the proximal convoluted tubuli (M.G.C.). In accordance with the results of other authors a definite age linkage was established. High incidence rates and strong M.G.C. reaction are found mainly in patients above 50 years of age. The age factor can be regarded, however, only as a constitutional basis in which arteriosclerotic changes may play the leading part. This is proved by the absence of M.G.C.s in cases of instantaneous death in aged individuals. Anoxia was found to be the decisive factor in the production of the phenomenon with this reaction being confined to individuals dying in a state of oxygen deficiency. Patients having received oxygen therapy for some time prior to death show a lower numerical and quantitative incidence of M.G.C. reaction than control groups not having received treatment. It is postulated that these findings constitute, on the basis of acid-base metabolism, a link with published experimental studies in which animals on a diet of excess inorganic phosphates as well as those kept in a state of potassium deficiency presented a M.G.C. reaction similar to the one discussed here.

OBSERVATIONS IN EXOGENOUS HEMOCHROMATOSIS APPARENTLY DUE TO MULTIPLE TRANSFUSIONS. Irving Graef and (by invitation) Benjamin S. Gordon, Walter Newman, Renzo G. Olivetti, and Bernard Klein, New York University-Bellevue Medical Center, and USVA Bronx Hospital, New York, N.Y.

Abstract. Five instances of "exogenous" hemochromatosis have been studied. Pathologic evidence in all, supported by chemical evidence of Fe content in 3, unequivocal clinical evidence in one, and equivocal clinical features in 3, warranted the pathologic diagnosis of hemochromatosis, indistinguishable from the spontaneous "endogenous" form. In all, large numbers of blood transfusions and hematinic therapy had been given to correct a refractory anemia over periods of 1 to 8 years. In 3, the anemia was idiopathic, pseudo-aplastic; the fourth had lymphatic leukemia with a refractory anemia. The fifth had scleroderma with refractory hypoplastic anemia.

At necropsy, grossly nodular pigmentary cirrhosis, with hepatomegaly and histologic features of fibrosis, bile duct proliferation and extensive Fe deposits in bile duct epithelium, parenchymal cells, and macrophages were found, with siderofibrosis of the pancreas. Fe pigment was demonstrated around the coiled glands of the skin in 4. Typical Fe deposits were found in the zona glomerulosa of the adrenal glands, thyroid epithelium, salivary glands, distal convoluted tubules of the kidney, and the myo-

cardium. In 2 instances in which sections of the anterior pituitary gland were made, intracellular Fe pigment was found in epithelial cells and macrophages; in these it was found likewise in the ependymal cells of the choroid plexus. Deposits in the villi of the small intestine were found in 2; prostatic epithelial deposits in 3/4 males; in gastric glandular epithelium in 3/5 cases; gonadal deposits consisted of Fe pigment in macrophages in 1/3 cases. Atrophy or fibrosis with reduced spermatogenesis was found in 2/3 cases where sections of testes were made. Splenic involvement was studied in 3; the others had had splenectomy. Moderate macrophage deposits were present in 3, and capsular or trabecular deposits were present in 2. Available lymph nodes in all 5 contained large amounts of Fe pigment within macrophages. Bone marrow was hypoplastic in one; erythrogenesis appeared intact in the others. Myelopoiesis was increased in these 4 as well, with lymphocytic proliferation in the one instance of lymphocytic leukemia. Smooth muscle contained no Fe pigment. Striated muscle in only one case contained scanty intracellular deposits. Hemofuscin granules were identified in smooth muscle of the gastrointestinal tract in 2/5 cases, and in the venous walls in a third instance.

For comparison five cases were chosen with similar disorders and refractory anemia in which equally large numbers of transfusions and hematinic therapy had been given. Four were males. The fifth, a female, died with disseminated lupus erythematosus. Pathologic evidence of Fe deposit was not associated with fibrosis in the liver or pancreas. Adrenal and thyroid involvement was negligible, being absent in some or scanty in others. Traces of Fe pigment were found in the anterior pituitary parenchymal cells of one of 2 glands studied. Myocardial involvement was virtually absent. The skin was unaffected. Chemical evidence in the second group provided values that overlap some of those found in the group with fibrotic changes in the liver, pancreas, and with the more extensive endocrine glandular or myocardial deposits.

The causes of the development of the complete picture of hemochromatosis in some cases and its absence in others with hemosiderosis are obscure. The many factors involving the metabolism, absorption, and excretion of Fe need investigation in this problem.

EXPERIMENTAL ERYTHROBLASTOSIS FETALIS. Aaron Kellner and Emily F. Hedal (by invitation), New York Hospital-Cornell Medical Center, New York, N.Y.

Abstract. A pair of allelic blood group factors bearing a striking similarity to the Rh-Hr factors in man has recently been identified in the blood of rabbits. Numerous observations have shown that these factors, designated G and g, are present in the red cells but not in the tissues or body fluids, that they are transmitted genetically from parents to offspring, and that they can stimulate the formation of isoantibodies and give rise to erythroblastosis fetalis under suitable experimental conditions. The injection of red cells containing either of these factors into rabbits lacking it resulted in most instances in the development of isoantibodies (anti-G and anti-g) specific for rabbit red cells containing the factor injected. Both agglutinating and coating antibodies were present in the serum of such injected animals. The coating antibodies, which were generally of higher titer than the agglutinins, were identifiable either by means of an anti-globulin test (Coombs test) using guinea-pig anti-rabbit-globulin serum or by means of specific cells modified by the action of trypsin. The antibodies have proved to be potent hemolysins and capable of fixing complement.

Rabbits may become immunized also to one or the other of these factors transplacentally during pregnancy. Thus, G-negative female rabbits (gg) mated to homozygous G-positive males (GG) had fetuses that were all G-positive (Gg), and in 5 of 10 such matings the mothers developed specific anti-G antibodies in their serum. Furthermore, the anti-G and anti-g antibodies, whether formed as a result of pregnancy or following the injection of red cells, proved capable of crossing the placenta into the fetal circulation and affecting the fetuses *in utero* if their red cells contained the appropriate antigen. When the mother's antibody titer was low, the

fetuses were usually born alive; their red cells, however, were in most cases coated with specific antibody, as was shown by a positive anti-globulin test and also by the fact that antibody could be eluted from the coated fetal cells. When the mother's antibody titer was high, the fetuses were often born dead and macerated; if born alive, they frequently died shortly after birth. There were 13 litters born to does with antibody titers of 1 to 64 or higher, and of 105 fetuses, 85 died. Again, antibody from the mother was regularly found coating the red cells of such fetuses, and it was demonstrable also in their serum. In addition, hydrops fetalis, necrosis of the liver, and enhanced extramedullary hematopoiesis were observed in the fetuses from isoimmunized mothers. Antibodies in considerable titer were identified in the milk of immunized mothers on many occasions.

HEMOGLOBIN-DERIVED AND IRON-STORAGE PIGMENTS IN CELLS: MICROSPECTROSCOPIC ANALYSIS. Robert C. Mellors, Memorial Center, New York, N.Y.

Abstract. An attempt is being made to characterize the absorption spectra and other properties of certain pigments as they occur normally and abnormally in tissue cells. The analysis is first applied to submicrogram quantities (order of 10^{-6} μgm.) of relatively pure materials such as the hemoglobins, hematin, bile pigments, ferritin, and hemosiderin, and to oxidation, reduction, and metallic derivatives of some of these compounds. A study is then made of pigments as they occur in individual tissue cells, particularly when there is a preponderance of one or another substance. Spectra in the visible region are recorded for the following substances in cellular samples of the order of 10 μ in thickness and 4 μ^2 in cross-sectional area: the hemoglobins of the red blood cell and other locations; the bile pigments in the liver and elsewhere; hemosiderin in reticulo-endothelial cells; and crystals of ferritin in smears of fresh liver and spleen. A finding of practical utility is made with respect to the tubular casts of hemoglobinuric and cholemic nephrosis: the pigments in either case have strong blue-violet absorption (Soret band), but the bile pigments are differentiated by the formation (with Schlesinger's reagent) of a zinc complex which has a bright red fluorescence.

IRON DEPOSITS IN THE BRAIN COMPARED WITH SIMILAR DEPOSITS IN SPLEEN, LIVER, LUNGS, AND OTHER ORGANS, AND THEIR RELATIONSHIP TO MENTAL DISEASES AND AGE. George Strassmann, Metropolitan State Hospital, Waltham, Mass.

Abstract. Two forms of iron deposits can be demonstrated in the brain by histochemical methods; one type is hemosiderin within phagocytic elements formed after hemorrhages; the other form of iron ("tissue iron") is visible only after special stains, situated around blood vessels, in vessel walls, and nerve cells of the extrapyramidal system. Both types of iron deposits are increased in general paresis, in patients with cerebral arteriosclerosis, and generally in all patients over 60 years old. The amount of iron in the brain was compared with that in spleen and liver, usually rich in iron deposits, and in other organs, such as lungs, pituitary body, thyroid gland, adrenal gland, and blood vessel walls. Frequently a parallelism existed between a large amount of iron in the predilected areas of the brain and the iron content of the spleen and liver; in other instances the amount of iron in the brain and these organs was different. Contrary to expectations, iron in alveolar phagocytes was often missed, despite cardiovascular disturbances, whereas coal pigment was always present. The iron deposits in other organs than brain, spleen, or liver could be identified as hemosiderin originating from former hemorrhages. In the nervous part of the pituitary body, iron deposits were a common finding. The increased iron content in the brain may be due to the large amount of iron necessary for the respiration of the nerve cells and also to the frequency of circulatory disturbances occurring in elderly patients, leading to increased deposits of hemosiderin and tissue iron in the areas of the basal ganglia, not typical for any special psychosis. The formation of hemosiderin after hemorrhages in the human brain and in human organs (myocardial and pulmo-

nary infarction) is slower than in tissues and organs of small animals (mice, rabbits) and 5 to 6 days are required as a rule before hemosiderin can be discovered in phagocytes.

THE MUSCLE TISSUE OF THE LUNG IN CHRONIC PULMONARY DISEASE. Averill A. Liebow, William E. Loring (by invitation), and Warren L. Felton, II (by invitation), Yale Medical School, New Haven, Conn.

Abstract. Muscle tissue is found in great abundance in the lung in many types of chronic pulmonary disease. It is especially prominent in pulmonary emphysema, even when that condition is focal and presumably in an early stage of development. Several sources of this muscle can be identified: (1) The walls of the distal respiratory passages including, in notable degree, the alveolar ducts. Here the muscle is so situated that, in spasm, it can contribute to the trapping of air distally. (2) The lymphatics, including those of the pleura. (3) The blood vessels, especially enlarged bronchial arteries. The latter in chronic pulmonary disease often possess not only very large lumina but also extremely thick muscular walls from which great sheets of large, smooth muscle cells extend into the surrounding parenchyma. Muscular proliferation and hypertrophy may be so great as to restrict or even obliterate the lumina of these vessels. In this way a large bronchial arterial collateral circulation can ultimately be obliterated. This occurs especially when the surrounding parenchyma becomes densely scarred.

PONTINE HEMORRHAGE IN INTRACRANIAL HYPERTENSION. William S. Fields (by invitation) and Béla Halpert, Baylor University College of Medicine and Veterans Administration Hospital, Houston, Texas.

Abstract. A study was made of 43 encephala with morphologic evidence of intracranial hypertension due to supratentorial space-occupying lesions to determine the frequency of hemorrhage into the pons and adjacent midbrain and to investigate the mechanism that produces it. The increased intracranial pressure was due to intracranial hemorrhage in 20, to primary intracranial neoplasm in 14, to metastatic growths in 8, and to brain abscess in one. Gross and microscopic studies disclosed varying degrees of hemorrhage into the substance of the pons or midbrain in 17 of the 43 brains. The hemorrhages usually were multiple and were either in or adjacent to the midline of the midbrain or the pontine tegmentum rather than in the base. They were more extensive in patients in whom intracranial hemorrhage was due to trauma than in those with vascular hypertension or aneurysm; they were least extensive in those with primary intracranial neoplasm. The clinical data disclosed that pontine or midbrain hemorrhage occurred only in those patients in whom there was a sudden change in intracranial hydrodynamics; in patients in whom the intracranial pressure had built up gradually, no such hemorrhage developed. The signs and symptoms associated with pontine or midbrain hemorrhage were: sudden coma, tachycardia, hyperthermia, respiratory distress, hypertension, and fixed pupils. The sudden change in intracranial hydrodynamics as brought about by ventriculography, craniotomy, or lumbar puncture caused herniation through the tentorial incisura resulting in hemorrhage into the pons and adjacent midbrain. It is believed that this occurs more often than herniation of the cerebellum and medulla oblongata into the foramen magnum and that it is of greater consequence.

THE NATURAL HISTORY OF THE LESIONS IN SARCOIDOSIS. H. J. Barrie and A. Bogoch (by invitation), Banting Institute, Toronto, Canada.

Abstract. The classical focal granuloma of sarcoid is formed initially by a process of active cellular proliferation. This stage is prominent only in early biopsies or in necropsy material when death has occurred early in the disease. Among 30 necropsied cases of sarcoidosis the proliferative phase was found in 8, and in 6 of these there was also an interstitial mononuclear pneumonitis. Among these 8 cases were found,

also, diffuse destruction of the parenchyma and mononuclear cell infiltration in the heart, liver, and central nervous system. Concentration on the focal granuloma of sarcoidosis has so far caused the importance of the diffuse inflammation to be overlooked. The nature of the initial inflammatory phase was discussed.

Fibrosis of the lungs in long-standing sarcoidosis is the result of three processes:

1. The direct result of the initial inflammatory phase.
2. The involvement of blood vessels by the granuloma leading to secondary ischemia.
3. The regression of focal granulomata leading to peripheral fibrosis.

As long as the granuloma remains stable it protects against fibrosis. The focal aggregates of epithelioid cells in sarcoidosis as well as in all the other granulomata can be regarded as a mechanism for isolating certain types of foreign material from contact with the fixed tissue cells.

SARCOIDOSIS AND HISTOPLASMOSIS. Lalla Iverson and Henry Pinkerton, Armed Forces Institute of Pathology, Washington, D.C., and University of St. Louis, St. Louis, Mo.

Abstract. Three fatal cases of histoplasmosis with disseminated sarcoid-like lesions were reported, serving as evidence for the view that subacute or chronic histoplasmosis may, in some instances, exhibit the clinical and pathologic features commonly regarded as diagnostic of sarcoidosis. Sarcoid-like lesions were present in the lymph nodes, lung, spleen, liver, and other organs. In all 3 cases the cause of death was bilateral mycotic caseation of the adrenal glands. Periodic acid stains showed in the caseous material huge numbers of histoplasma organisms which could not be identified in sections stained in the routine way.

The evidence strongly suggests that these cases are examples of uncomplicated histoplasmosis and that the commonly accepted view that sarcoidosis is an etiologic entity may be erroneous. The occurrence of sarcoid-like lesions in histoplasmosis may be explained as follows: Fungi entering the blood stream from active focal lesions in the lungs, adrenal glands, or elsewhere may be carried to lymph nodes, skin, or other organs. Because of better tissue immunity, they may be rapidly killed, setting free antigen which results in the production of non-caseating lesions of the sarcoid type. If the active focal lesions heal or become quiescent, a prolonged and relatively benign clinical course with periodic exacerbations might result. Histochemical studies show that dead fungi without internal structure can be identified only by special stains which color their capsules. The 3 cases further emphasize the possibility of confusion of histoplasmosis with tuberculosis. Even a classical lesion such as caseous adrenal necrosis should not be assumed to be of tuberculous etiology unless sections of the involved tissues are studied by means of special stains and the specific organism demonstrated.

PITUITARY NECROSIS IN ROUTINE NECROPSIES.* Alfred Plaut, Veterans Administration Hospital, Topeka, Kans.

Abstract. The anterior lobe of the pituitary gland, in routine necropsy material, may contain small or fairly large areas of necrosis, and this is not a rare occurrence. The process obviously is not dependent upon embolism, thrombosis, or other mechanical factors. The observations were discussed in relation to the problems of Simmonds' disease and the Sheehan syndrome.

STUDIES ON THE NATURE AND PATHOGENESIS OF MUSCULAR DEGENERATION IN CORTISONE-TREATED RABBITS. John T. Ellis, New York Hospital-Cornell Medical Center, New York, N.Y.

Abstract. Muscle lesions have been noted by others in cortisone-treated animals,

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

but they have not been described in detail and their pathogenesis has not been considered. In experiments undertaken for other purposes, we have recently observed widespread degeneration of skeletal muscles following the administration of massive doses of cortisone to rabbits. Observations on the nature and pathogenesis of the muscle changes are here reported in brief. Voluntary muscles were procured from the trunks and extremities of 28 young rabbits given cortisone, 10 mg. per kg. per day, for periods of 7 to 21 days or longer; all regularly displayed degenerative changes that became more severe with the passage of time. When examined 7 to 14 days later, large segments of many of the fibers in each muscle were swollen to three or four times their normal size; these were much paler in fixed and stained preparations than the adjacent unchanged segments within the same sarcolemmal sheaths and their structure was homogeneous and devoid of identifiable myofibrillae; occasionally lipids and calcium salts, separately or together in small amounts, were deposited in them. The protoplasm of the swollen segments was actually necrotic, for it was more or less promptly phagocytosed by mononuclear cells, which soon became conspicuous within the stretched and sometimes ruptured sarcolemmal sheaths and not infrequently formed syncytial giant cells as they encircled the necrotic material. After 21 days, some 40 to 80 per cent of the muscle fibers visible in any given field displayed the segmental swelling and necrosis just described. In addition, atrophy of the uninvolved fibers was quite widespread. Fibrosis was not conspicuous, though interstitial edema with occasional mononuclear inflammatory cells was regularly seen.

The necrotic changes, when viewed in the large, were seen to resemble closely those described in Zenker's degeneration of skeletal muscle, in potassium deficiency, in vitamin E and other deficiency states, and in muscles of mice infected with the Coxsackie virus. No such abnormalities were observed in the cardiac and involuntary muscles of the cortisone-treated rabbits, and skeletal muscles of 65 control and 5 starved rabbits likewise failed to disclose them.

To learn more about the cortisone-induced lesion, chemical analyses were made on necrotic muscles procured from 6 rabbits treated with the drug for periods of 10 to 22 days, and on normal muscles from 8 controls. Half of each muscle was used for histologic study, the rest being analyzed for water, lipids, N, Na, and K. When compared with the controls, the necrotic muscles exhibited slight increases in content of water (average, 78.2 per cent; controls, 77.3 per cent) and larger increases in lipids (average, 2.2 per cent; controls, 0.7 per cent); they contained similar amounts of N (average 14.66 per cent on fat-free samples); and they had lost significant amounts of K (average, 39.9 mEq per 100 gm. of fat-free muscle; controls, 54.0) while gaining corresponding amounts of Na (average, 25.3 mEq per 100 gm. of fat-free muscle; controls, 9.0). Cardiac muscle from the cortisone-treated rabbits did not display these chemical alterations. Their significance is being studied further.

SYMPOSIUM ON THE PATHOLOGIC ASPECTS OF REACTIONS TO IONIZING RADIATION

CELLULAR CHANGES AFTER IONIZING RADIATION: HISTOCHEMICAL STUDY AND ULTRA-VIOLET MICROSCOPY. S. C. Sommers, M. W. Holt (by invitation), and Shields Warren, Cancer Research Institute of New England Deaconess Hospital, Boston, Mass.

Abstract. Frozen-dried dry-mounted tissues of animals exposed to ionizing radiation have been examined histochemically and with the polaroid color-translating ultra-violet microscope. X-rays, a cyclotron beam, and radioactive isotopes all have previously been shown to produce intranuclear vacuoles, which are large in radiosensitive and small in radioresistant cells. The vacuoles stretch and rupture nuclear membranes in radiosensitive tissues, but are extruded from resistant liver cell nuclei

without lethal effects. Histochemical stains have shown loss of nuclear desoxyribose nucleic acid post-irradiation, but have failed to identify the vacuole contents. Particular attention has been directed to intranuclear vacuoles as demonstrated by ultra-violet microscopy. This method permits detection of the presence of compounds which absorb in the spectral range of 2400-2800 Å. With the ultra-violet microscope the intranuclear vacuoles and pyknotic changes produced by radiation showed variable absorption at 2600 Å. Some damaged nuclei demonstrated marked concentration of substances with ultra-violet behavior characteristic of nucleoproteins and nucleic acids. By this technic, histochemical studies of irradiated nuclei are shown to be accurate in localizing nucleoproteins.

SOME SIMILARITIES OF ACTION BETWEEN IONIZING RADIATION AND SULFHYDRYL ENZYME INHIBITION IN VIVO. Samuel P. Hicks (by invitation),* New England Deaconess Hospital and Harvard Medical School, Boston, Mass.

Abstract. Current evidence suggests that acute ionizing radiation damage to tissues results from alteration of easily oxidized prosthetic groups of enzymes, especially sulfhydryl. This led to a comparison of *in vivo* effects of radiation with certain forms of metabolic inhibition, especially those that affect sulfhydryl enzymes. A useful test object is the developing mammalian nervous system, specifically that of fetal (second and third trimester) and newborn mice or rats. Developing nervous tissue cells, which may conveniently be called neuroblasts, are easily killed by ionizing radiations, the lower dose level for neuroblasts of fetal mice *in utero* being of the order of 40 r. (200 kv., 10 m.a., inherent Al filter 3 mm., 25 cm., about 50 r. per minute total body irradiation to the mother).

Pregnant and newborn animals were exposed to a variety of agents and forms of acute metabolic inhibition: asphyxia in nitrogen, insulin hypoglycemia, cyanide, malononitrile, azide, dinitrophenol, iodosobenzoate, chlormercuribenzoate, mapharsen, fluoracetate, aminopterin, triethylene melamine (TEM), methyl-bis-beta-chlorethyl amine (nitrogen mustard), plasmocid, phenylmercuric nitrate, fluoride, and iodoacetate. Selective necrosis of neuroblasts was caused by chlormercuribenzoate, mapharsen, and iodosobenzoate, which are known sulfhydryl reagents (substances that react with sulfhydryl). Triethylene melamine and the nitrogen mustard, also highly reactive toward sulfhydryl, were especially destructive to neuroblasts. Aminopterin, an antimetabolite, behaved similarly. Fluoracetate and plasmocid were also effective, the action of the former being to block citrate metabolism at the pyruvate (3 carbon) and acetate (2 carbon) levels. Acute asphyxia of pregnant animals, asphyxia of newborn animals for 20 to 45 minutes, cyanide hypoglycemia and the other agents were ineffectual against neuroblasts. Neither fluoride nor iodoacetate damaged neuroblasts, though the latter is fairly specific *in vitro* for a glycolytic sulfhydryl enzyme, phosphoglyceraldehyde dehydrogenase. Hypoglycemia and anoxia rarely did accomplish brain damage, but the damage was to relatively mature nerve cells, not to the radiosensitive neuroblasts. The mercury, iodoso-, and arsenic sulfhydryl reagents differed somewhat from radiation by also damaging muscle but not hemopoietic tissues.

The radiosensitive neuroblast might be tentatively characterized as being metabolically relatively independent of aerobic oxidation of glucose (failure of effect of anoxia, hypoglycemia, iodoacetate, and fluoride) and perhaps dependent on 2 and 3 carbon fragments (effect of fluoracetate). It might further be characterized as highly dependent on a system of sulfhydryl enzymes concerned with protein synthesis (aminopterin, sulfhydryl reagents, mustard, TEM).

To test these hypotheses further, combinations of sulfhydryl enzyme inhibitors (sequential blocking) and ACTH and cortisone, whose actions are suspected to be in part an inhibition of sulfhydryl enzymes, were given. ACTH and cortisone produced mild to severe necrosis of neuroblasts and also damaged some of the more

* By special invitation of the Program Committee.

mature nerve cells in both fetuses and newborn animals. When chlormercuribenzoate, mapharsen, and iodoacetate were given simultaneously, each in one-third effective dosage, they damaged neuroblasts; iodoacetate alone, or the other two agents together each in one-third dosage, were ineffective. This remarkable additive effect suggests that, although these reagents are fairly specific *in vitro* for certain sulfhydryl enzymes, they must have some blanket effect on other sulfhydryl enzymes.

The differences and similarities between the actions of low doses of radiation and some of these chemical agents may reside in relative specificities of actions. Radiation and the highly reactive mustard and TEM may blanket the whole gamut of sulfhydryl enzymes by disturbing only slightly the oxidation-reduction equilibrium of the sulfur hydrogen bonds. On the other hand, the sulfhydryl reagents such as chlormercuribenzoate may affect one enzyme strongly and the others just sufficiently to imitate the radiation effect in part.

Two further approaches (January, 1952) are in progress to learn by analogy whether radiosensitivity is closely related to a dependence on an integrated system of sulfhydryl enzymes. One aims to determine by enzymatic blocking *in vivo* whether the capacity for anaerobic metabolism in the newborn is related to a dependence on a system of sulfhydryl enzymes. The other is concerned with the relation of radiosensitivity of mouse neuroblastoma to its destruction by mapharsen and chlormercuribenzoate.

LESIONS IN SWINE EXPOSED TO TOTAL BODY GAMMA RADIATION FROM AN ATOMIC BOMB SOURCE. John L. Tullis, Baldwin G. Lamson (by invitation), and Sidney C. Madden,* National Naval Medical Center, Bethesda, Md.

Abstract. Forty-two swine were simultaneously exposed to a single lethal dose of total body gamma radiation from an atomic bomb source. Twenty-four of these were serially sacrificed in pairs at intervals from 4 hours to 6 days after irradiation. The remaining 18 swine died spontaneously on the fifth, sixth, and seventh days. Radiation-induced gross pathologic changes included reduction in size of thymus and spleen, gradual depletion of the bone marrow, congestion and inflammation of the gastro-intestinal tract, and hemorrhage into various organs. Pulmonary edema, bacterial invasion, and increased hemorrhage in the gastro-intestinal tract and other organs were conspicuous in animals dying spontaneously.

Microscopic alterations resembled those produced by 600 r. total body x-radiation. Lymphocytes, erythroblasts, myeloblasts, spermatogonia, and selected epithelial cells of the small intestine showed the most extensive changes. Destruction of lymphocytes in all lymphoid organs existed in the animals sacrificed at 4 hours and was generally most extensive in the next animals killed at 7½ hours. By 1½ days the excess débris had been removed by phagocytosis, and lymphocyte repopulation was evident after the second day following exposure. A progressive decrease in bone marrow cellularity was first recognized in the second sacrifice group at 7½ hours. Cells of the erythropoietic series were first reduced in number followed by myelopoietic cells and megakaryocytes in that order. Only reticular cells and a few more differentiated cells remained in animals dying spontaneously. Necrosis of epithelium of the small intestine was minimal, early, and completely repaired when examined at 25½ hours. Testes showed a loss of spermatogonia after 1½ days. Capillary congestion, edema, hemorrhage, and growth of bacterial colonies were present in many organs in all animals dying spontaneously.

PATHOLOGIC CHANGES IN PARABIOTIC RATS AFTER HEMI-ADRENALECTOMY AND X-RADIATION. Joshua L. Edwards (by invitation) and Sheldon C. Sommers, Duke University, Durham, N.C., and Cancer Research Institute, New England Deaconess Hospital, Boston, Mass.

Abstract. Parabiotic rats were used to study possible indirect effects of total body

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irradiation due to circulating substances. One hundred and twelve Slonaker rat pairs and suitable controls were subjected to 200 kv. x-radiation of the right partner, with shielding of the left partner, at doses of 400, 800, 1200, or 1600 r. The animals were sacrificed at intervals from 1 hour to 29 days, and 21 different tissues examined histologically, particularly for radiation changes. In addition, 74 of the pairs had both adrenal glands removed from one partner before irradiation. Presence of effective vascular connections was proved with T 1824 dye and P^{32} labeled erythrocytes. Leukocyte counts were used as a check of radiation effect. No evidence was found for radiation damage to the protected member caused by circulating substances from the irradiated partner. Parabionts had increased resistance to radiation of one member, as shown by less marked leukopenic responses compared to controls. Circulating leukocytes from the non-irradiated member supported the radiated partner's leukocyte counts.

Certain incidental observations were of interest. Formation of accessory adrenal cortical tissue partly compensated for hemi-adrenalectomy. The tissues most damaged by irradiation—lymphoid, hematopoietic, intestinal, and gonadal—showed brief periods of hyperplasia in the non-irradiated partners. This growth seemed due to stimuli from tissue destruction and to be not specific for radiation. It was particularly noted in hemi-adrenalectomized parabionts. Anterior pituitary and thyroid glands, and the pancreas also showed reactive alterations. The thymus behaved like other lymphoid tissues. Degenerative and proliferative cardiac, renal, and hematologic interreactions, observed more often in hemi-adrenalectomized parabionts, were not inhibited by radiation.

THE EFFECT OF WHOLE BODY X-RADIATION UPON CELL DIVISION IN RADIOSENSITIVE AND RADIORESISTANT TISSUES. R. Bland Williams, Jr., Robert P. deLong, and Julian J. Jaffe (all by invitation), Naval Medical Research Institute, Bethesda, Md.

Abstract. Male Sprague-Dawley rats weighing between 175 and 225 gm. were subjected to whole body x-radiation 48 hours after partial hepatectomy. The degree and duration of mitotic inhibition produced in the regenerating liver and epithelium of the small intestine by 450 and 900 r. total body x-radiation (200 KVP therapy machine) were compared. The lower dose constituted approximately an $LD_{50/30}$; 900 r. was a supralethal dose. The number of mitotic figures occurring during 4-hour intervals following irradiation was studied by the use of colchicine. The first period investigated was from 1 to 5 hours after irradiation. Data were also obtained from 5 to 9, 9 to 13, 21 to 25, 45 to 49, 69 to 73, and 93 to 97 hours.

From 1 to 5 hours after 450 r. and 900 r., mitotic activity was so profoundly reduced in both tissues that it was impossible without careful counts to distinguish any difference between the two levels. During this period the liver was more sensitive to mitotic inhibition, but showed no evidence of nuclear fragmentation, which was present to a high degree in the epithelium of the small intestine. In contrast to the very slight difference in early response to these levels of radiation, there was a profound difference in the return to a normal mitotic rate at all subsequent time intervals in both the bowel and liver. From 45 to 49 hours after 450 r. the mitotic index in the small bowel epithelium was 35 times as high as it was during this period after 900 r. The number of mitotic figures in the liver exceeded control levels from 45 to 49 hours after 450 r. After 900 r. the mitotic rate never returned to control levels in either tissue.

Some aspects of the pathogenesis of the mitotic inhibition have been explored. Three thousand r. whole body x-radiation greatly reduced the number of mitotic figures in the liver 48 hours after partial surgical removal, even when the animal was exposed to radiation prior to partial hepatectomy—that is, with the liver in the resting state. Five hours after 450 r. and 68 hours after 3,000 r., no evidence of any

indirect effect of radiation injury in the form of cell damage or mitotic inhibition was encountered in a 3 to 4 cm. loop of small bowel, exteriorized and shielded with ⅛ inch of lead. Placing an arterial clamp across a similar loop of intestine for 5 minutes before and during the administration of 450 r. total body radiation resulted in partial protection of the clamped loop compared with the rest of the animal's bowel 5 hours after irradiation. Complete regeneration of small bowel epithelium has been reported by others following radiation of the abdomen only with 1,000 r. In view of our results with 900 r. total body radiation, various shielding procedures, including protection of the spleen, are under investigation.

COMPARATIVE HISTOLOGIC EFFECTS OF 400 KILOVOLT AND 23 MILLION ELECTRON VOLT X-RAYS ON THE LONG BONES OF YOUNG ALBINO RATS. Earl W. Cauldwell and Granville A. Bennett, University of Illinois College of Medicine, Chicago, Ill.

Abstract. A histologic study was made of femurs and tibias of young albino rats after exposure of the knee joint area to varying x-ray doses from different energy sources. Parallel series, totaling 508 animals between 28 to 30 days of age, were irradiated by groups at doses ranging from 200 r. to 5000 r. at 400 kv. and 23 mev., respectively. The severity of damage to the epiphyseal cartilage was roughly proportional to the air dose as measured in roentgens from 400 r. to 2000 r. at 400 kv., and from 600 r. to 3300 r. at 23 mev. At higher doses maximum tissue changes were observed, which suggested a saturation effect. Initial damage to chondrocytes occurred in the zone of cellular multiplication and linear orientation (zone II). Irreparable damage to the epiphyseal cartilage produced by maximal doses was expressed by degenerative and necrotizing changes in chondrocytes and matrix, and the subsequent development of a bony lamina across the metaphyseal surface of the cartilage. In a few cases a "secondary" epiphyseal cartilage plate was established near the metaphyseal end of the diaphysis, but gave no evidence of contributing to longitudinal growth. Complete sterilization of the epiphyseal cartilage was not accomplished even at 5000 r. with either energy source, as viable cells persisted in zone I, and irregular nodular proliferation of cartilage frequently occurred at the periphery of, and on, the epiphyseal surface of the plate.

Articular cartilage was significantly damaged with a dose of 3000 r. at 400 kv. More spectacular alterations in its structure occurring after 45 days were related to changes arising from disturbed bone mechanics: flattening of epiphysis, malalignment of growth axis, and compression fractures in metaphysis.

The histologic changes in animals comprising the two energy series could be correlated with the measurable inhibition of growth observed in the gross long bones. The latter quantitative data have been used as one source of biologic reference to the relative effectiveness of 400 kv. and 23 mev. x-rays when the ionization produced by each has been measured in air with a conventional technic.

NUCLEAR CHANGES FOLLOWING IRRADIATION OF TUMORS. Austin M. Brues (by invitation),* Argonne National Laboratory, Chicago, Ill.

Abstract. Gigantic nuclei containing large nucleoli, vacuoles, and finely dispersed chromatin have been observed frequently in tumors and other tissues that have undergone irradiation. Although swelling of nuclei can occur almost immediately after a single irradiation, the most strikingly enlarged nuclei are observed later, following continuous or intermittent treatment. By the use of point sources of beta radiation embedded in a carcinoma, the enlargement of nuclei has been followed over the course of 7 days and correlated with the suppression of mitotic division that takes place. The results indicate that gradual enlargement of nuclei to 5 or 10 times their normal volume occurs when the dose rate is above 40 rep per hour, and suggest that it takes place through a continuation of growth while mitosis is inhibited.

* By special invitation of the Program Committee.

MORPHOLOGY OF TRANSPLANTABLE PITUITARY TUMORS INDUCED BY RADIOACTIVE IODINE AND THE ASSOCIATED SECONDARY CHANGES. J. Furth,* Oak Ridge National Laboratory, Oak Ridge, Tenn.

Abstract. All five strains of transplantable pituitary tumors induced in mice by I^{131} are composed of chromophobe cells with only a rare, faintly basophil cell. Nevertheless, they all secrete thyrotropic and gonadotropic hormones. The changes indicative of these secretions in tumor-bearing hosts and in bioassays were demonstrated. These include hyperplasia of the thyroid gland with features of grave hyperthyroidism, extensive ovarian changes as seen in pregnancy tests, and hyperplasia of Leydig cells. A novel finding consists of tremendous cystic dilatation of the common duct associated with hyperplasia of ductal epithelium. This change, frequently leading to spontaneous rupture, occurs terminally in every animal bearing a grafted tumor. These and other characteristic changes were illustrated and the mechanism of induction of neoplasia in endocrine organs by ionizing irradiations was discussed.

LEUKEMIA IN HUMAN EXPOSEES TO THE HIROSHIMA AND NAGASAKI ATOMIC BOMBS.† B. Black-Schaffer and (by invitation) S. Kambe, S. Matsuoka, Z. Watanabe, and W. J. Wedemeyer, Jr., Atomic Bomb Casualty Commission of the National Research Council, Hiroshima, Japan, Nagasaki University Medical School, Nagasaki, Japan, and Hiroshima University Medical School, Hiroshima, Japan.

Abstract. During 1949, 1950, and 1951, about 50 instances of leukemia among individuals exposed to the atomic bombs of Hiroshima and Nagasaki were brought to the attention of the staffs of the Atomic Bomb Casualty Commission and its associated Japanese physicians. Eighteen were necropsied. The first figure represents an incidence of approximately 13.0 per 100,000 population. Comparing this to the best available data from non-exposed Japanese of the same cities, this reflects a highly significant increase. Experimental, and limited clinical, experience have indicated that leukemia and other blood dyscrasias may be produced by exposure to externally applied ionizing energy. This study presents the hematopoietic lesions and sequelae in our series arising 4 to 6 years after exposure to the atomic bombs.

THE INHIBITORY EFFECT OF BETA RADIATION (P^{32}) ON FIBROPLASIA IN RAT SKIN. C. C. Lushbaugh (by invitation) and John B. Storer (by invitation), University of California and Los Alamos Scientific Laboratory, Los Alamos, N. Mex.

Abstract. The abdominal skin of 60 adult albino rats was irradiated with 28,000 rep of beta radiation. This dose was delivered by 1 inch plastic disks impregnated with radiophosphorus, at an average rate of 4500 rep per hour. Surgical incisions were made through the abdominal wall in both normal and "burned" areas the day before, the same day, or the day after exposure to radiation, respectively, in three groups of 20 rats each. Animals were then killed at daily intervals after the exposure and histologic sections made of the wounds in an irradiated and non-irradiated area. It was found that the early features of wound healing, *viz.*, hyperemia, edema, exudation of polymorphonuclear leukocytes, exudation of lymphocytes, and appearance of polyblasts and macrophages, were not prevented by local radiation, but that differentiation of fibroblasts, fibroplasia, and capillary proliferation were prevented except in the preperitoneal connective tissue. Semiserial sections of the wounds showed that this inhibition was limited to the area of the exposure and did not spread from it. By exposing 40 additional rats in groups of 10 rats each to 3500, 7000, 14,000, and 28,000 rep and preparing histologic sections of similarly made surgical wounds it was found that the depth to which this inhibition extends through the body wall

* By special invitation of the Program Committee.

† Not presented.

was proportional to the dose of radiation delivered. It was concluded from these observations that the inhibition of fibroplasia and capillary proliferation by radiation is an important factor in the delayed healing of radiation ulcers and surgical wounds in irradiated areas.

THE PATHOLOGY OF THE ACUTE RADIATION SYNDROME IN MAN. Hermann Lisco,* Argonne National Laboratory, Chicago, Ill.

Abstract. The pathologic changes occurring in the course of the acute radiation syndrome in man have been studied in 2 patients who accidentally sustained total-body exposures to a lethal dose of ionizing radiation consisting principally of gamma rays and neutrons without concomitant heat or blast components. The patients died on the 9th and on the 24th day after exposure. In general the pattern of injury was that familiar from earlier studies on the effects of ionizing radiation on normal tissues of animals and man. Thus the hematopoietic organs, the intestinal tract, the skin, and the testes suffered the greatest damage. Although there were, at the time of death, varying degrees of regeneration and repair in some tissues in both patients, there is evidence to show that the viability of some regenerating cells was considerably impaired and modified. An attempt is made to explain this phenomenon, at least in part, on the basis of demonstrable cytologic abnormalities in the tissues of both patients. At least one of the observations that were made in the course of this study is not in harmony with present concepts of radiosensitivity and recovery from radiation injury. In one patient the regeneration of the lymphatic tissue had already commenced on the ninth day after exposure, while the bone marrow was still totally aplastic at that time.

RELATION OF HEMORRHAGE TO HEMATOPOIESIS, BLOOD COAGULATION AND MORTALITY AFTER TOTAL BODY X-IRRADIATION. Robert L. Rosenthal (by invitation), Naval Radiological Defense Laboratory, San Francisco, Calif.

Abstract. The pattern of hemorrhage following total body x-irradiation was studied in relation to blood coagulation, blood count, and bone marrow content. The guinea-pig was used because it develops marked hemorrhage following irradiation. Two hundred and forty-four English short hair, male and female guinea-pigs were used. Single doses of 250 kv. total body x-irradiation were given as follows: 87 received 200 r., 30 day lethal dose (L.D.) of 10 to 20 per cent; 47 received 400 r., L.D. 60 to 80 per cent; 40 received 600 r., L.D. 100 per cent; 70 served as controls. Animals were sacrificed in groups of 3 to 7 for each dosage at the following times after irradiation: 4 hours, 1, 3, 6, 9, 13, 16, 20, 23, 28, and 30 days. Control animals were sacrificed with each group.

Under light nembutal anesthesia the chest was opened, the heart exposed, and 10 cc. of blood taken by cardiac puncture. Care was exercised to avoid contamination with tissue juice. Red cell, white cell, differential, platelet, and reticulocyte counts, erythrocyte sedimentation rate and hematocrit reading were taken. Clotting time, heparin clotting time, and the quantitative measure of clot retraction by the electric resistance technic were performed on whole blood. Prothrombin time, recalcified plasma clotting time, and toluidine blue titration tests were performed on oxalated plasma. Smear and imprint preparations were made from femoral bone marrow. Post-mortem examinations were performed upon all animals. The degree of hemorrhage in each organ was graded as follows: 0 (none), 2 (slight), 4 (moderate), or 6 (marked). In each animal, the scores of the organs were totaled and the result defined as units of hemorrhage. Hemorrhage was also evaluated in this manner in animals which died spontaneously following irradiation. The 70 control animals showed no hemorrhage.

Hemorrhage was slight until the 9 to 13 day period after irradiation when it rapidly

* By special invitation of the Program Committee.

reached its height, which was more marked in the animals receiving higher doses. Hemorrhage was found to occur in conjunction with the fall in platelets below 30,000, prolongation of the heparin clotting time, and absence of clot retraction as measured by the electric resistance technic. The thrombocytopenia was in turn closely related to the disappearance of megakaryocytes from the bone marrow. As bone marrow regenerated and the platelets increased, a rapid decrease in hemorrhage was noted. In the 200 r. animals, the score of hemorrhage decreased from 24.1 units to 6.6 in the 9th to 13th day, while in the 400 r. guinea-pigs there was a decrease from 27.3 units to 4 in the 13th to 16th day. The hemorrhage occurred in order of decreasing frequency in the stomach, lymph nodes, large intestine, small intestine, lungs, heart, adrenal glands, kidney, and bladder. The 200 and 400 r. animals, which died spontaneously in the 9 to 16 day period after irradiation, showed significantly more hemorrhage than the 200 and 400 r. animals sacrificed in this period. This difference was not apparent in the 600 r. animals. A marked anemia developed concurrently with the height of the hemorrhage and gradually disappeared with bone marrow regeneration. Skin purpura resulted from trauma among animals in contact with each other and was avoided by keeping them in individual cages.

These studies indicate for the guinea-pig a close relationship between hemorrhage, bone marrow function, platelet level, and anemia. The observation that dead animals had more pronounced hemorrhage than those sacrificed points to hemorrhage as a factor in mortality. However, the significance of hemorrhage seems to differ from one species to another. The rat, mouse, and rabbit develop less hemorrhage and have a higher 50 per cent lethal dose of total body x-ray than the guinea-pig, dog, and man.

STUDIES OF THE CLOTTING DEFECT IN DOGS FOLLOWING TOTAL BODY IRRADIATION. George D. Penick (by invitation), University of North Carolina School of Medicine, Chapel Hill, N.C.

Abstract. Hemorrhage is one of the widely recognized features of the syndrome resulting from total body exposure to ionizing irradiation. However, there is not complete agreement regarding the basis of its development. Studies of the clotting mechanism have demonstrated prolongation of the whole blood clotting time and thrombocytopenia, but no significant reduction in plasma fibrinogen, prothrombin, or accelerator globulin.

In the current study, 10 healthy normal dogs were subjected to lethal doses of irradiation and the blood coagulation defect studied. Alteration in the clotting mechanism consisted of a progressive impairment in the prothrombin conversion rate as measured by the thrombin tide and the two-stage prothrombin utilization tests. Accordingly, two of the main factors responsible for speed of prothrombin conversion—platelets and antihemophilic factor (AHF)—were studied quantitatively. A progressive thrombocytopenia developed in all dogs and closely paralleled the retardation of prothrombin utilization. AHF levels were assayed by a procedure recently developed in this laboratory and were found to be maintained within normal limits throughout the post-irradiation period. These data were confirmed by transfusing plasma from irradiated dogs into hemophilic dogs with resulting correction of the deranged clotting of the hemophilic recipients. In additional experiments *in vitro*, correction of the clotting defect in irradiated dog's blood was obtained by the addition of hemophilic whole blood, which was deficient in antihemophilic factor but adequately supplied with formed elements. The addition of washed normal dog platelets to plasma from an irradiated dog had a similar corrective effect, producing a thrombin tide comparable to that of fresh normal plasma under the same circumstances. These findings indicate that the blood from irradiated dogs at the height of the clotting defect is potentially capable of clotting at a normal rate, provided that an adequate number of platelets is present.

These data were collected as part of a collaborative study with the Naval Medical Research Institute, Bethesda, Md.

CORRELATION OF BONE MARROW INJURY WITH MORTALITY IN DIFFERENT SPECIES OF IRRADIATED MAMMALS. George Brecher and E. P. Cronkite (by invitation), National Institutes of Health and Naval Medical Research Institute, Bethesda, Md.

Abstract. Guinea-pigs and Sprague-Dawley rats of comparable weight were exposed to single whole body irradiation of 400 r. (200 KVP). This dose killed 95 per cent of guinea-pigs, usually between the 12th and 14th day, and was sublethal for rats. Ten guinea-pigs killed on the 10th day showed almost complete aplasia of the bone marrow, while 10 rats killed on the same day showed complete or nearly complete regeneration of their marrows. Similarly, none of 40 dogs exposed to a uniformly fatal dose of 600 r. (2,000 KVP), which were killed or died between the 4th and 18th day, showed any evidence of bone marrow regeneration, while 8 rabbits exposed to the same dose, which is sublethal for this species, and killed on the 7th and 10th day showed extensive marrow recovery. Regeneration of the lymphoid tissue was delayed in the dog as compared to the rabbit. However, guinea-pigs and rats exposed to 400 r. showed similar degrees of recovery of the lymph nodes on the 10th day. The clear-cut correlation between lack of bone marrow recovery and death from ionizing radiation in different species lends further support to the concept that, at the dose levels used, the sequelae of pancytopenia induced by bone marrow injury are the principal, though not necessarily the only, causes of death.

THE GENERAL PATHOLOGY OF RADIATION REACTION. Shields Warren (referee *), Cancer Research Institute of New England Deaconess Hospital, Boston, Mass.

MODIFICATION OF ACUTE IRRADIATION INJURY IN MICE AND GUINEA-PIGS BY BONE MARROW INJECTIONS: A HISTOPATHOLOGIC STUDY. C. C. Congdon, D. Uphoff, and E. Lorenz (all by invitation), National Cancer Institute, National Institutes of Health, Bethesda, Md.

Abstract. A total body exposure of 900 r. invariably kills mice of strains C_3H_b, A and L, and hybrid LAF_1 within 21 days. The 30 day-LD_{50} for mice of these strains varies from 560 r. to approximately 650 r. Inbred guinea-pigs, for which the 30 day-LD_{50} is approximately 400 r., die when exposed to 550 r. total body dose and usually within 21 days. However, if the mice exposed to 900 r., or the guinea-pigs exposed to 550 r., are injected with homologous bone marrow after irradiation, mortality decreases significantly. The mortality of irradiated mice in the four strains varies from 0 to 30 per cent following intravenous injection of homologous bone marrow. Intraperitoneal injection of bone marrow is less effective in some strains, and the mortality range is from 25 to 90 per cent. In guinea-pigs, injection with homologous bone marrow is also highly effective in preventing death from irradiation injury whether the route of injection be intravenous, intracardiac, or intraperitoneal. Mortality rate ranges from 10 to 50 per cent depending on the route of injection. Proliferation of injected cells lodging in hematopoietic tissues is a possible explanation of recovery of animals when homologous bone marrow is used. However, cellular repopulation of the bone marrow by injected cells is an unsatisfactory explanation since heterologous bone marrow, injected intravenously, also gives protection to mice irradiated with a total body dose of 900 r., mortality being reduced to approximately 60 per cent after this treatment. The donor animals for these mice were either mice of a different strain or inbred guinea-pigs. Histologic studies have been made from animals killed serially after receiving irradiation only, and from animals receiving bone marrow injections following irradiation. Normal animals with and

* By special invitation of the Council.

without bone marrow injections were also studied. Nearly total destruction of hematopoietic and lymphatic tissues occurred in the days immediately following irradiation in all animals. Treated animals showed beginning regeneration of depleted tissues approximately 5 to 6 days after irradiation in contrast to non-treated irradiated animals which showed no significant recovery of blood-forming tissues. Peripheral blood studies correlated well with the histopathologic changes. Development of bone with marrow spaces was found in the peritoneal cavities of guinea-pigs following intraperitoneal injection of bone marrow alone. The significance of this observation was discussed.

THE EFFECT OF LOW DOSAGE TOTAL BODY IRRADIATION ON THE HEMATOPOIETIC SYSTEM OF DOGS. H. J. Van Baaren (by invitation), F. J. Ragaz (by invitation), and D. M. Angevine,* University of Wisconsin School of Medicine, Madison, Wis.

Abstract. The effects of total body irradiation with low dosage (10, 25, 50, and 100 r.) of x-rays over a prolonged period were studied on a group of 38 thoroughbred cocker spaniels and beagles, male and female, weighing from 7 to 11 kg. A control group was used in which the animals were subjected to identical procedures as the experimental group but without irradiation. The effect of irradiation on the hematopoietic system was studied by frequent complete blood counts in association with bone marrow studies. The bone marrow samples were taken by a Turkel needle, the use of which enabled us to make repeated bone marrow studies upon the living animal.

Special attention was given to the increase in the number of band neutrophils which appeared in the circulation soon after irradiation. Bone marrow samples taken after irradiation showed a definite increase in the number of band forms present in the marrow. No such changes were noted in the control group. Total body irradiation in a dosage of 25 r. to 100 r. apparently leads to an increase in white cell maturation in the bone marrow followed by a release of band neutrophils into the peripheral blood. Animals which received the doses as low as 10 to 25 r. did not show a lymphocytopenia. Such correlative studies of bone marrow sections in relation to the peripheral blood have been a valuable means of detecting minor changes resulting from the effects of irradiation after the administration of small doses of x-rays.

EFFECTS OF RADIOACTIVE GOLD ON THE RAT LIVER. Simon Koletsky, Western Reserve University, Cleveland, Ohio.

Abstract. Internal radiation of the rat liver was studied by means of intravenous injection of radioactive colloidal Au^{198}. The latter is stable and insoluble and when given intravenously is removed from the circulation by the reticulo-endothelial system. About 90 per cent of the injected dose is deposited in the liver within the Kupffer cells. Single massive doses of Au^{198} were employed and these provided radiation to the liver ranging from approximately 12,000 to 48,000 equivalent roentgens. The result was marked hepatic cell necrosis with distortion of architecture. Most of the surviving hepatic parenchyma was converted to typical giant cells which may represent an abortive type of regeneration. The lesion resembled cirrhosis but was not accompanied by significant fibrosis. No injury was noted to Kupffer cells, biliary epithelium, or vascular bed. There was evidence of hepatic dysfunction after 1 month, frequently associated with jaundice and ascites. Most of the animals died between 2 and 5 months after the radioactive material was injected.

HISTOLOGIC EFFECTS OF THERAPEUTIC DOSES OF RADIOACTIVE IODINE ON THE THYROID GLAND OF MAN. Austin L. Vickery (by invitation), Massachusetts General Hospital, and Harvard Medical School, Boston, Mass.

Abstract. This paper constitutes a portion of a combined study of the functional

* By special invitation of the Program Committee.

and histologic effects of radioactive iodine on the human thyroid gland done in collaboration with B. M. Dobyns, F. Maloof, O. Cope, and E. M. Chapman. Although radioactive iodine has been used for 10 years in the treatment of thyrotoxicosis, few observations have been published on the nature of the cellular changes following irradiation. Such histologic sequences are of considerable importance because the major concern in the use of radioactive isotopes is the question whether cancers will develop in future years. No such reports have appeared in the literature in relation to radioactive iodine.

During the past 10 years, over 400 patients with thyrotoxicosis have been treated with radioactive iodine in the Thyroid Clinic of the Massachusetts General Hospital. Of these, samples of thyroid tissue were obtained from 25 patients, ranging from 19 days to 8 years after therapeutic doses of I^{131} or I^{130} had been given for hyperthyroidism. Histologic examination of this material revealed a wide range of tissue response to radiation. While some of these glands showed no detectable radiation effect (possibly due to inadequate doses of the isotope or relative insensitivity of the cells to the delivered radiation), others exhibited a marked response with the parenchyma practically replaced with connective tissue. The outstanding cytologic abnormalities following radiation were noted in the nuclei of residual thyroid cells. These consisted of irregularities in nuclear size and shape, often associated with bizarre distortions of the chromatin pattern. These changes varied considerably from one area to another and even within the same follicle. The finding of such bizarre nuclear forms in cells comprising intact, colloid-containing follicles as long as 8 years following irradiation raises the issue of their significance. Autoradiographic studies have shown that areas of thyroid tissue containing many such atypical cells still have a capacity to take up tracer doses of radioactive iodine. This observation, with the preservation of cell outlines, suggests that such abnormal appearing cells are, to some degree, still viable and functional.

Two interesting questions are posed by these observations: (1) Are these cells the originally irradiated ones which have survived in an altered form; or (2) do they represent propagated mutant descendants of the original partially damaged cells? These questions are yet unanswerable, but their relationship and importance to the problem of potential neoplasia are obvious. In over 400 patients treated with radioactive iodine, no neoplasm of the thyroid gland traceable to radiation has developed; however, definite answers to the question of tumor formation must await more prolonged observation.

THE EFFECT OF X-RAYS ON THE VIRUS-HOST CELL RELATIONSHIP. F. S. Cheever (by invitation),* Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pa.

Abstract. Experiments have been carried out to investigate the possible effects of ionizing radiations on the virus-host cell relationship. X-rays have been used as the source of ionizing radiations, and the host rather than the viral agent has been irradiated. The course of St. Louis encephalitis infections in normal mice inoculated by several routes has been compared to that observed in mice irradiated at various intervals before or after inoculation of the virus. Similar experiments have been carried out employing the Powers strain of Coxsackie virus. By means of Bernkopf's de-embryonated egg technic a comparison has been made of the rate of multiplication of influenza A virus (PR-8 strain) in chorioallantoic membranes derived from normal chick embryos and in those obtained from embryos given non-lethal doses of x-rays prior to de-embryonation. Some of the results of these preliminary experiments are reported.

* By special invitation of the Program Committee.

THE RELATION BETWEEN MORPHOLOGIC AND IMMUNOLOGIC CHANGES CAUSED BY RADIATION. Frank J. Dixon and David W. Talmage (by invitation),* University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Abstract. The antibody response can be divided into an initial radiosensitive phase lasting only a few hours and a subsequent radioresistant phase lasting for the remainder of the period of antibody production. The first phase is completely suppressed by small amounts of radiation given before the antigen. In the rabbit 200 to 300 r. whole body radiation given 2 days to several weeks before an antigen prevents an antibody response. The morphologic changes resulting from this amount of x-radiation are minimal, transient, and limited chiefly to the lymphoid tissue, bone marrow, and gonads. Histologic recovery is complete within 1 to 2 weeks, long before recovery of the immune system. Radiation will not suppress the antibody response if given after exposure of the animal to an antigen. In the rabbit 400 r. whole body radiation given simultaneously with the antigen (bovine gamma globulin) or as much as 800 r. ($LD_{50/30}$) given 3 days after the antigen have little effect on the serum antibody levels which are maximum about 10 to 12 days after antigen injection. Yet these doses of radiation cause profound damage in many of the body tissues. Eight hundred r. destroys most of the lymphoid tissue and bone marrow leaving only the reticulo-endothelial cells and a few obviously injured lymphocytes or hematopoietic cells for 5 to 10 days. Significant histologic recovery takes several weeks. In several rabbits which received 800 r. and died of radiation injury, relatively normal serum antibody levels were maintained until death.

From these observations it appears that the initial phase of the antibody response is dependent upon some extremely radiosensitive process, the site of which cannot be determined histologically since it is destroyed by amounts of radiation too small to produce persistent, significant, morphologic change. The second phase, which involves the actual synthesis of antibody protein, is certainly not dependent upon any radiosensitive cells or tissues. Whether antibody synthesis in the non-radiated animal takes place in radiosensitive tissues cannot be determined, but this does not seem likely. Finally, in reference to the time-honored controversy about antibody production and lymphocytes, it can be said that the only possible rôle of lymphocytes in antibody production would be in the short initial phase and not in the actual synthesis of antibody.

THE EFFECT OF GAMMA RADIATION ON ANTIBODY PRODUCTION AND IMMUNITY. William M. Hale (by invitation) and Richard D. Stoner (by invitation),* Brookhaven National Laboratory, Upton, Long Island, N.Y.

Abstract. Data were presented to include the following:

1. The amount of gamma radiation from a Co^{60} source required to inhibit antibody production, as well as the effect on antibody present at the time of irradiation.
2. The duration of the effect of radiation on antibody formation.
3. The effect of gamma radiation on established immunity to infections by *Diplococcus pneumoniae, Trichinella spiralis,* and influenza virus.

EFFECTS OF X-RAY ON TRICHINA LARVAE. S. E. Gould, University of Michigan (U.S. Atomic Energy Laboratory) and Wayne County General Hospital, Eloise, Mich.

Abstract. Preliminary to studying the effects of x-irradiation on trichina larvae in pork, it was decided to determine (1) the dose of irradiation delivered to trichina larvae *in vitro* that would prevent experimental infection of trichinosis in rats, and (2) the lethal dose of irradiation for trichina larvae, and the time of death of larvae

* By special invitation of the Program Committee.

following their irradiation. The following results have been obtained. Rats fed trichinae irradiated with doses of 800 to 3500 r. develop relatively mild or extremely mild trichina infections, as judged by the numbers of larvae that were recovered from their muscles, compared with control rats fed non-irradiated larvae. When larvae irradiated with 5000 r. or over were fed to rats, no larvae were recovered from the muscles of the experimental animals. Doses of x-ray of 400 r. and up to 100,000 r. do not kill the larvae at once but seem to hasten death. The effects of various doses upon the survival rate were presented.

EFFECTS OF TOTAL BODY RADIATION ON A CHRONIC, LATENT INFECTION IN WHICH IMMUNITY IS NOT DEPENDENT ON THE SPLEEN (TOXOPLASMOSIS). J. K. Frenkel, L. Jacobs, and M. L. Melton (all by invitation), National Institutes of Health, Bethesda, Md.

Abstract. Several chronic infections, such as malaria, relapsing fever, and murine bartonellosis, are affected by splenectomy. The labile immune state is thought to be dependent on the integrity of the lymphoid-macrophage system, of which the spleen constitutes an important part. Since sublethal doses of total body radiation can severely damage this system, a subsequently relapsing bartonellosis might be essentially analogous to the effects of splenectomy, that is due to lymphoid-macrophage dysfunction alone. Chronic toxoplasmosis is not affected measurably by splenectomy, and it is therefore unlikely that the lymphoid-macrophage system plays an important rôle in maintenance of latency in immunity. Hence, any radiation effect on latent toxoplasmosis should be ascribed to additional damage.

Male, white, 5-weeks-old mice of the NIH-BS strain were infected subcutaneously with the low-virulent Ch strain of toxoplasma. Four weeks thereafter, one half were super-infected subcutaneously with the highly virulent RH strain, and the other half were reinjected with the Ch strain. At the age of 3 months, groups of infected Ch-Ch mice were irradiated with 300, 475, or 700 r., or were left as unirradiated controls, and compared with uninfected mice treated similarly. The irradiation factors were: 200 kv., 20 m.a., target distance 50 cm., 0.25 mm. copper and 0.51 mm. aluminum, output rate approximately 60 r. per minute. Similar numbers of infected and uninfected mice were irradiated in the same lucite chamber, holding 10 mice. Mice dying within 4 weeks are listed in the numerator, the total number of mice is expressed by the denominator.

	Deaths	
Radiation	*Ch-Ch*	*Uninfected*
None—splenectomized	1/10	—
None—not operated	1/10	—
300 r.	1/10	4/10
475 r.	7/10	0/15
700 r.	10/10	10/10

All deaths occurred within 13 days, with the majority between the sixth and eighth days. Several mice showed multiple bites and it is thought that some deaths were hastened by subcutaneous hemorrhages (especially in the uninfected 300 r. group). Sections from representative mice showed that all those infected initially still harbored toxoplasma pseudocysts in their brain. Lesions in the irradiated mice were consistent with those occurring after total body radiation alone; there was no toxoplasmic proliferation or lesion indicative thereof.

In order to examine progressive changes after radiation, half of the mice infected with both Ch and RH strains were irradiated with 300 r., and killed after 1, 4, 5, 7, 9, 11, and 13 days, together with unirradiated controls. Tissues were fixed and sectioned; all were found to be infected. Intact toxoplasma pseudocysts were present

in their brains unassociated with lesions. No differences were detected between the two groups in the reaction to their infection. The irradiated group showed lesions unassociated with toxoplasma and attributable to radiation alone.

No reactivation of toxoplasmosis occurred after sublethal and lethal radiation that caused marked lymphoid destruction, such as has been observed in certain other infections in which the spleen plays a rôle in immunity. Hence, the apparent increase in mortality is not due to toxoplasmic proliferation. Experiments are in progress to study the effects of cortisone and of nitrogen mustard on chronic, latent toxoplasmosis, since some of their effects mimic those found after radiation.

A Correlation of Morphologic with Metabolic Changes in Rat Skin Following Exposure to Beta (Sr^{90}) Radiation. C. C. Lushbaugh (by invitation) and D. B. Hale (by invitation), Los Alamos Scientific Laboratory, University of California, Los Alamos, N.Mex.

Abstract. Using a disk of Sr^{90} 5/8 inch in diameter, delivering 90 rep beta per second, 73 rats were given 14,000 rep and 44 rats were given 28,000 rep to the abdominal skin by contact with this plate. Erythema, followed by ulceration, developed after 4 days with 28,000 rep and after 6 days with 14,000 rep. The course of the acute ulcerative radiodermatitis was followed in the 14,000 rep exposures by killing rats every other day for histopathologic study. By the usual Warburg manometric methods oxygen consumption and glycolytic rate for the whole skin exposed to 14,000 rep or 28,000 rep and normal adjacent skin were determined at the same 2-day intervals for 15 days. This study showed, when the metabolic changes were correlated with the histologic changes occurring during the developmental period of the "burn" (6 days for the skin exposed to 14,000 rep), that both oxygen consumption and glycolysis were remarkably elevated, being 180 per cent of normal 2 days after exposure. The Q_{O_2} and Q_{CO_2}, both fell from this high level and were normal when early histologic degenerative changes were first seen in the epidermis at 6 days. They were 50 per cent of normal during the mid-period of ulceration at 8 to 10 days, and rose again to values above normal as epithelial regeneration began from the margin of the wound and fibroplasia occurred in the subcutaneous tissue under the "burn" at 12 to 14 days of age. This study would appear to indicate that if immediate enzymatic damage occurs in an intact organ as a result of exposure to ionizing radiation, as it has been demonstrated to occur *in vitro* with pure enzyme systems, other unknown changes must occur in these enzyme systems which lead to a prolonged enhancement of oxygen consumption and glycolysis which diminishes only as degeneration and necrosis develop. Reparative changes are likewise paralleled by a rise in oxygen consumption and glycolysis.

Some Autoradiographic Studies of the Distribution of P^{32} and S^{35} in Mammalian Tissues. Margaret W. Holt (by invitation) and Shields Warren,* Cancer Research Institute, New England Deaconess Hospital, Boston, Mass.

Abstract. The distribution patterns obtained in various mouse and rat tissues following subcutaneous injection of the P and S isotopes as $(P^{32}O_4)\equiv$ and $(S^{35}O_4) =$ ions were illustrated. The use of frozen dried tissue preparations and selective extraction technics has made it possible to obtain some information about the chemical form of the isotope as it is distributed within the tissues at various times following injection. P^{32} is concentrated in the small intestine at the base of the glands of the mucosa, whereas S^{35} is concentrated throughout the mucosa and excreted into the lumen. Other tissues such as the kidney, spleen, liver, lung, and brain show some differences in isotope distribution, but these are less striking than is true of intestine. The distribution of the two isotopes in the embryo is very similar, marked

* By special invitation of the Program Committee.

accumulation occurring in sites of cartilage formation. Their distribution in the placenta, however, is strikingly different, indicating different mechanisms of transfer. These patterns are of significance also in the evaluation of the given isotope in regard to effectiveness as a local source of irradiation and potential radiation damage.

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IMMUNITY TO TUMORS TRANSPLANTED IN THE BRAIN OF HETEROLOGOUS HOSTS. E. J. Eichwald, O. N. Rambo, and J. Henry (all by invitation), University of Utah College of Medicine, Salt Lake City, Utah.

Abstract. The brain of heterologous hosts has been found to be a suitable site for the growth of transplanted tumors. Tumors transplanted to this site grow faster and reach a larger size than those transplanted to the anterior chamber of the eye. Also, tumors transplanted to the brain occasionally grow progressively, and cause death of the host. In contrast, tumors transplanted to the anterior chamber invariably regress. This regression is assumed to result from the development of an immune state. In support of this theory is the failure of a second tumor to grow in the contralateral eye when transplanted at the time of regression of the first tumor.

The experiments reported were devised to determine why tumors grow better in the brain than in the eye. The following possibilities were considered: (1) A tumor transplanted to the brain fails to give rise to an immune response; (2) A systemic immune reaction may be produced but does not influence the growth of the tumor; (3) Factors other than immunity account for the difference in growth.

Mouse sarcoma 37 was transplanted to the brains of guinea-pigs. On the 10th day after transplantation the animals received anterior chamber transplants. On the 20th day the eyes were removed and sectioned serially. None of them showed growing tumor, while tumor was present in all control animals. This indicates that tumors transplanted to the brain give rise to an immune response. In a second experiment mouse sarcoma 37 was transplanted subcutaneously in guinea-pigs of one group, and to the anterior chamber in another group. On the 10th day both groups and a control group received intracranial transplants. On the 20th day the animals were sacrificed. Growing tumor was present in all animals of the control group, and absent in those with previous implantation into the subcutaneous tissue or the eye. Evidently systemic immune events influence the growth of tumors transplanted to the brain. It is concluded that factors other than immunity are the cause for the difference in tumor growth in the two transplant sites.

NATURAL RESISTANCE AND ACQUIRED IMMUNITY TO TUMORS TRANSPLANTED TO THE ANTERIOR CHAMBER OF HOMOLOGOUS HOSTS. E. J. Eichwald, H. Y. Chang, and M. Hattori (all by invitation), University of Utah College of Medicine, Salt Lake City, Utah.

Abstract. To determine the extent to which strain barriers are lowered by use of the anterior chamber as a transplantation site, several mouse tumors were transplanted to various strains of mice. Equal numbers of each strain received subcutaneous or anterior chamber transplants. The barriers of all strains were lowered; establishment of tumors in the anterior chamber occurred in a large majority of mice of all foreign strains, while this did not take place after subcutaneous inoculation. The extent to which strain barriers were lowered differed with the tumor and the strain. In no instance were strain barriers abolished. When the tumors grew progressively the survival periods were prolonged, and metastatic dissemination did not occur. However, the tumors often regressed, presumably due to the development of a resistant state. It is surmised, therefore, that the natural resistance of a foreign host to a tumor is by-passed by use of the anterior chamber, but that the barrier of acquired immunity is not overcome. Transplantation to the anterior chamber thereby

becomes a convenient means of testing the strength of the immune response of a host to a tumor. Subcutaneous transplantation is less suitable for this purpose since many tumors fail to become established and cannot demonstrate the development of a resistant state. Administration of cortisone, therefore, does not necessarily increase the incidence of progressive growth of subcutaneously transplanted tumors, while it raises the incidence following anterior chamber transplantation.

TUMORS PRODUCED BY METHYLCHOLANTHRENE IN THE DUCK: PAPILLOMA, SQUAMOUS CELL CARCINOMA, AND HEMANGIOMA. R. H. Rigdon, University of Texas Medical Branch, Galveston, Texas.

Abstract. White Pekin ducks apparently have been used only once for the study of neoplasms with chemical carcinogens. Duran-Reynals observed one fibrosarcoma in a group of 52 ducks injected with methylcholanthrene. In this study a 0.25 per cent solution of methylcholanthrene in acetone was applied daily for 33 to 66 days to the skin beneath the wing of ducks varying in age from 10 days to adult. Papillomas appeared approximately 1 month after the experiments were begun and hemangiomas appeared in the subcutaneous tissues of the same birds approximately 2 months after the experiments were started. Squamous cell carcinomas occurred in the skin of these ducks during this time. No neoplasms appeared following the local application of acetone to the skin of ducks. Thirty-six ducks were injected subcutaneously or intramuscularly with methylcholanthrene in sesame or mineral oil. These birds were sacrificed at intervals. The majority were examined between 18 and 22 months. A hemangioma was found in the pectoral muscle at the site of injection in 2 ducks. Several of the ducks developed large non-neoplastic tumors at the site of the injection.

All papillomas disappeared spontaneously. Malignant changes were present in the base of some. None of the birds had a malignant tumor when they were sacrificed 6 to 12 months after the experiments were begun. A majority of the hemangiomas likewise disappeared from the subcutaneous tissue.

THE OCCURRENCE IN TARRED RABBIT SKIN OF MINOR, ALMOST IMPERCEPTIBLE, NEOPLASTIC CHANGES. Peyton Rous and Stanfield Rogers (by invitation), the Rockefeller Institute for Medical Research, New York, N.Y.

Abstract. Previous work in this laboratory has shown that most of the tumors tar elicits from rabbit epidermis require aid for their survival—which tarring supplies. It brings into existence numerous latent tumor cells which never form growths unless stimulated. The question arises whether some hidden neoplastic elements may not be so poorly endowed as to multiply into visibility only on prolonged urging. The non-carcinogenic stimulus of wound healing was used to find out. Holes nearly 1 cm. across were punched through rabbits' ears, repeatedly tarred months before, and when they had filled with new tissue all of this save a peripheral zone was punched out if no tumors had appeared. This was done again and again after healing, only the original zone remaining. Its cells were thus kept multiplying throughout many months, and sooner or later some of them slowly formed small growths. None appeared carcinomatous; most were low papillomas having epithelium that looked merely hyperplastic, while others were of kinds unreported previously. All arose as radial entities on the peripheral zone, extending inwards as the tissue next them did so. Their radial shape showed they had originated from cells permanently changed prior to the first punching. The majority soon retrogressed unless repunching was done. The experiments have disclosed neoplastic deviations from the normal so slight in their expressions as to become manifest only under exceptional circumstances. They must be reckoned with when considering the nature of neoplastic change.

CARCINOLYTIC ACTION OF A SERIES OF NEW BIOLOGICALLY SIGNIFICANT DIHYDROTRIAZINES. Sidney Farber and (by invitation) Israel Diamond, George Foley, and Edward J. Modest, the Children's Cancer Research Foundation and the Department of Pathology, Harvard Medical School, at the Children's Hospital, Boston, Mass.

Abstract. A new series of biologically significant dihydrotriazines (analogues of 4,6-diamino-1-(p-chlorophenyl)-1,2-dihydro-2,2-dimethly-s-triazine hydrochloride) synthesized by one of us (E.J.M.) represents a hitherto unreported class of compounds with anti-vitamin and anti-malarial activity. Under appropriate microbiologic metabolic conditions these compounds exhibit antagonism to folic acid, niacin, riboflavin, and citrovorum factor. Significant carcinolytic changes have been produced against sarcoma 180 and both lymphatic and myelogenous leukemia in the mouse.

HISTOCHEMICAL AND MORPHOLOGIC DIFFERENTIATION OF INDUCED TUMORS OF THE LIVER IN RATS. Harlan I. Firminger (by invitation) and Ambadas S. Mulay (by invitation), National Institutes of Health, Bethesda, Md.

Abstract. Tumors induced in the livers of rats fed p-dimethyl-aminobenzene-1-azo-1-naphthalene (DAN) were morphologically and histochemically similar to those induced with p-dimethylaminoazobenzene. Both carcinogens induced hepatomas, adenocarcinomas, and mixtures of these two types. A few animals fed DAN also developed undifferentiated carcinomas. The adenocarcinomas occasionally contained areas of squamous differentiation and frequently were composed, at least in part, of mucus-secreting cells similar to those seen in intrahepatic non-neoplastic pathologic bile ducts. Such expressed potentialities of the cells of the adenocarcinoma favor its origin from bile duct epithelium and the term cholangioma seems applicable to this group of tumors.

FACTORS CONCERNED IN THE DEVELOPMENT OF EXPERIMENTAL TESTICULAR TUMORS IN RATS. Gerson R. Biskind and (by invitation) Donald E. Bernstein and Morton S. Biskind, Mt. Zion Hospital, San Francisco, Calif.

Abstract. In 1945 we reported the production of experimental testicular tumors in the Sherman strain of rats. These tumors were induced by the transplantation of a newborn rat testis into the spleen of an adult castrate rat. At that time the unusual histologic similarity between the ovarian and testicular tumors was noted, but no elucidation of the histogenesis of the latter tumor was given. In subsequent investigations with the Long-Evans strain, ovarian tumors occurred in all animals employed; but, to our surprise, in this strain testicular tumors were never obtained even though the experiment was attempted in 95 animals.

The present report (a) gives strong evidence that the testicular tumor develops from the interstitial cells and (b) presents two experimental methods for inducing testicular tumors after intrasplenic transplantation in the Long-Evans strain.

(a) It has been possible to trace the formation of the tumor from scattered foci of interstitial cells which ultimately coalesce into a sharply demarcated mass. Remnants of the spermatic tubules are often present around the periphery of the mass. At this stage the tumor is almost indistinguishable from the luteoma that follows the transplantation of an ovary into the spleen of a castrate rat. Later the testicular tumor may undergo histologic changes that resemble a granulosa cell tumor or an arrhenoblastoma.

(b) The first method employed for the induction of the tumor was the administration of exogenous hormones. Pregnant mare's serum injected three times weekly induced luteoma formation in the testicular transplant in 3 animals in periods of 292 to 329 days. The administration of luteotrophin, testosterone, cortisone, desoxycorticosterone, and progesterone had no effect. The second successful method was

adrenalectomy. Three animals having a testicular transplant were adrenalectomized and maintained on saline solution. The 2 survivors were sacrificed after 440 days and each showed striking tumor development. Although careful search was made for adrenal cortical tissue, none was found in one animal and in the other only one remnant 1 mm. in diameter was present.

It is of unusual interest that the testicular and ovarian experimental tumors are almost indistinguishable histologically. It is of great importance that in a hitherto tumor-resistant strain of rats, two experimental methods of tumor induction have been successful, each of which involves a basic hormonal alteration rather than the application of a foreign carcinogen.

PROPERTIES OF TRANSPLANTABLE METHYLCHOLANTHRENE-INDUCED SARCOMAS IN THE NEWT. Charles Breedis, University of Pennsylvania School of Medicine, Philadelphia, Pa.

Abstract. A preliminary report of the first successful induction of a transplantable neoplasm in a cold-blooded vertebrate by a chemical carcinogen was given last year. Two of 34 adult *Triturus viridescens* that had been injected into the forelimb with methylcholanthrene crystals developed sarcoma at the site of injection within 16 months. These tumors proved readily transplantable to other *T. viridescens* by material containing living cells. One tumor is now in the 7th passage, the other in the 3rd. Both sarcomas are deeply invasive growths that penetrate blood vessels and destroy nerves, muscle, and bone. They have been transplanted to the limbs, body wall, tail, and peritoneal cavity. They tend to kill the host by invasion of neighboring structures rather than distant metastases; only one of more than 100 transplanted growths has metastasized. When animals inoculated with sarcoma were kept at two different temperatures, 20° and 30° C., the neoplastic growth was more rapid at the higher temperature. Animals resistant to one inoculation sometimes could be successfully inoculated later. Attempts to transmit the tumors with glycerinated, dried, or filtered material have failed. When the tumor was rendered bacteria-free by antibiotics it remained transplantable. These observations are evidence for the belief that the intact tumor cell is necessary for propagation of the neoplasm.

The large size and small chromosome number ($2n = 22$) of Triturus cells make them excellent material for cytologic study of neoplasms.

AN ELECTROPHORETIC STUDY OF THE ORIGIN OF THE ABNORMAL PLASMA PROTEINS IN MULTIPLE MYELOMA. Gail Lorenz Miller, Clark E. Brown, Elizabeth Eshelman Miller, and Edward S. Eitelman (all by invitation), the Institute for Cancer Research, the Lankenau Hospital Research Institute, and the Lankenau Hospital, Philadelphia, Pa.

Abstract. An electrophoretic study was made of the plasma and extracts of tumor of a typical multiple myeloma subject. Tumor was obtained at necropsy from the vertebrae, ilium, and lymph nodes. Control tests were made with corresponding normal plasma and normal tissues, and with tumor from a subject with myelogenous leukemia. Vertebral marrow obtained from a multiple myeloma subject who had undergone urethane treatment also was investigated. A large, sharp peak, having a mobility somewhat lower than that of normal gamma globulin, characterized the electrophoretic pattern of the plasma and the tumor extracts of the test subject. In special studies, an effort was made to exclude the effects of blood or lymph as possible contaminants in the tumor extracts. The characteristic sharp peak was not observed in any of the control experiments. It was also absent in the extract of the marrow of the urethane-treated multiple myeloma subject, due, presumably, to the effect of the urethane in inhibiting the growth of the myeloma cells. The results appear to support the view that myeloma cells are the site of formation of the abnormal plasma proteins of multiple myeloma.

THE PRECIPITIN TEST FOR BENCE JONES PROTEIN: REPORT ON THE SEROLOGIC TESTS ON URINE OF 1000 INDIVIDUALS, EMPLOYING ANTIBODY PREPARED BY THE ADJUVANT TECHNIC. Fred C. Collier (by invitation) and Paul Jackson (by invitation), Ohio State University Medical School, Columbus, Ohio.

Abstract. The need for a specific, sensitive test for Bence Jones protein has long existed. The chemical procedures employed to demonstrate this substance are not sufficiently precise to give positive results when the protein is present in minimal concentration. Of the available methods for demonstrating the presence of the protein, the most acceptable, from the standpoint of sensitivity, are both physical in nature—the use of the ultracentrifuge and the electrophoretic apparatus. Even these methods, however, are not sufficiently delicate to give positive results if the protein be present in concentrations less than 0.5 gm. per cent. A serologic test, by far the most sensitive for unknowns, was suggested by Hektoen in 1921.

Preparation and Titration of Antibody. Antibodies to Bence Jones protein were induced in rabbits in two fashions. Bence Jones protein obtained from 4 patients with multiple myeloma was purified and crystallized. Each protein was injected intravenously into 4 rabbits. Several courses of injections were necessary to produce titers of 1:180. Use of a modification of the adjuvant technic described by Freund produced in 4 weeks titers of 1:920 in rabbits subjected to intravenous and intramuscular immunization. These figures refer to the highest titers obtained in homologous antigen-antibody reactions. Heterologous antigen-antibody reactions occurred in all cases, suggesting the immunologic similarity of the six Bence Jones proteins investigated. Precipitin reactions were not observed in tests of all 24 rabbit anti-Bence-Jones sera with normal human gamma globulin. This same situation obtained in testing rabbit anti-gamma-globulin serum with solutions of any of the six Bence Jones proteins.

Clinical Application. The high titer immune sera were utilized in "screening" patients for possible excretion of Bence Jones protein. Positive precipitin tests were recorded on the urine of the following groups:

33 patients, previously undiagnosed, with negative chemical tests for Bence Jones protein in the urine, but in whom later diagnostic studies proved the disease to be multiple myeloma.

20 patients, previously diagnosed as suffering from multiple myeloma, but with negative chemical tests for Bence Jones protein in the urine.

3 patients, with negative chemical tests for Bence Jones protein in the urine, in whom subsequent laparotomies showed solitary plasmacytomata.

1 patient with acute lymphatic leukemia.

Negative tests were recorded on the urine of the following groups:

608 patients selected at random in whom diagnostic studies revealed diseases other than blood dyscrasias or the myeloma-plasmacytoma group.

158 normal individuals without known illness of any kind.

177 patients diagnosed as having leukemia: 51, acute lymphatic leukemia; 30, chronic lymphatic leukemia; 10, acute monocytic leukemia; 4, lympholeukosarcoma; 52, acute myelogenous leukemia; 30, chronic myelogenous leukemia.

No known false positive or false negative tests were observed.

THE SURVIVAL OF HUMAN TUMOR TISSUE ON THE CHORIOALLANTOIC MEMBRANE OF THE CHICK EMBRYO. Nathan Kaufman (by invitation), L. C. Prieto, Jr. (by invitation), Earl J. Mason (by invitation), and Thomas D. Kinney, Cleveland City Hospital and Western Reserve University, Cleveland, Ohio.

Abstract. The survival of human tumor tissue on the chorioallantoic membrane of the developing chick embryo was studied. The tissue was obtained from biopsy specimens and small fragments measuring 1 to 3 mm. in diameter were placed on the chorioallantoic membrane near blood vessels. The egg was then sealed. Incubation

of the eggs at the time of inoculation varied from 6 to 12 days. Six tumors were studied on eggs without further transplantation, and fragments of each tumor were well maintained up to 11 days when the experiment was terminated. In addition, 20 tumors were placed on chorioallantoic membranes of developing eggs and were then transferred to new eggs. Sixteen of these tumors were maintained during the primary explantation period; of these, 5 survived one transplantation and 2 survived two transplantations. Serially transplanted tumors did not fare as well as the original explants. In an attempt to adapt the tumors to the chick embryo, 11 tumors were grown in tissue culture first, and then transplanted to the chorioallantoic membrane. Ability to survive in the new environment after previous *in vitro* cultivation, as compared with direct explantation, seemed not to be enhanced.

It was noted also that the chorioallantoic membrane reacted to the foreign tissue with the formation of epithelial pearls, fibroblastic proliferation, keratinization, and stratification of the membrane epithelium. The reactive patterns were not constant but their absence could not be correlated with any particular type of tumor. It appeared, too, that the tumor was first maintained on the chorioallantoic membrane by means of a plasmatic circulation. Later the tumor became vascularized and chick erythrocytes could be seen in vascular channels in the human tumor tissue.

SELECTIVE UPTAKE OF RADIOACTIVE SULFUR BY HUMAN CHONDROSARCOMAS. Raymond G. Gottschalk (by invitation) and Herbert C. Allen, Jr. (by invitation), Radioisotope and Medical Research Units and Laboratory Service, Veterans Administration Hospital, and Baylor University College of Medicine, Houston, Texas.

Abstract. The ability of cartilage of rats to retain sulfur and to incorporate it into chondroitin sulfate, and the frequent similarity of metabolism of homologous normal and malignant tissues suggested that radioactive sulfur might be taken up by chondrosarcomas. Tracer doses of S^{35} were given as sodium sulfate to 2 adult patients. One had a chondrosarcoma of the ilium with multiple metastases; tissues were obtained by biopsy after 65 hours, and at necropsy 11 days after the injection. The other patient, with a slowly growing chondrosarcoma of the tibia, received S^{35} 50 hours prior to amputation. Radiation counts on the exposed surfaces of the tissues, radioautographs, and determinations of activity of the total sulfur, isolated as barium sulfate, indicated about the same degree of concentration of S^{35} in normal cartilage, in the chondrosarcomas, and in the metastases. The amount of tracer element was lower in the viscera and soft tissues. The concentration fell rapidly in the blood, while the cartilaginous tissues retained higher levels of S^{35}. These findings were discussed in relation to possible diagnostic or therapeutic applications.

DEVELOPMENT OF ACUTE LEUKEMIA IN HUMAN ADULTS. Matthew Block (by invitation) and Leon O. Jacobson (by invitation), University of Chicago, Chicago, Ill.

Abstract. Observations on the development and histogenesis of acute leukemia in the human adult are extremely rare. In the last 2 years 9 adults have been studied by means of serial blood counts and serial biopsies of the marrow and, in some cases, of the liver and spleen during periods of up to 18 months before the acute leukemia became manifest. Seven were menopausal females, 4 of whom had multiple allergies varying from asthma to drug idiosyncrasy. All had thrombopenia and neutropenia which in some was noted as long as 1 year preceding development of leukemia. Each patient was anemic for prolonged periods and this anemia usually was not benefited by transfusions, indicating the possibility that abnormal hemolysis antedated the leukemic state.

Hypercellularity and maturation arrest in the granulocyte series were seen months prior to leukemic metaplasia of the marrow. In approximately half the patients the marrow was solidly cellular during the pre-leukemic phase. One patient had an

atrophic and then an aplastic marrow for several months. The marrow then became solidly leukemic in a period of only 6 days. Liver and spleen did not undergo leukemic transformation as early as did the marrow and in some patients at necropsy were less involved than the marrow.

THE HISTOGENESIS OF MALIGNANT MIXED TUMORS OF MÜLLERIAN ORIGIN (MIXED MESODERMAL TUMOR OF THE UTERUS). Wallace H. Clark, Jr. (by invitation), William H. Sternberg, and Robert C. Smith (by invitation), Tulane University and Charity Hospital of Louisiana, New Orleans, La.

Abstract. Uterine tumors containing a variety of sarcomatous and carcinomatous elements have been described under a variety of terms including mixed mesodermal tumor, sarcoma botryoides, and carcinosarcoma. During the 6-year period ending in January, 1952, 13 such tumors have been seen in the Charity Hospital at New Orleans. Each tumor contained at least two varieties of sarcoma and 10 contained carcinomatous elements as well. The sarcomatous elements included endometrial sarcoma, leiomyosarcoma, rhabdomyosarcoma, fibrosarcoma, and chondrosarcoma. The carcinomatous elements were endometrial adenocarcinoma, papillary cystadenocarcinoma, tubal adenocarcinoma, and epidermoid carcinoma.

Our findings indicate that the tumor arises from derivatives of the Müllerian anlage, most frequently the endometrial stroma. Favoring this view are the following observations:

1. Endometrial stroma closely resembles, histologically, the primitive mesenchyme surrounding the embryonic Müllerian duct.
2. In 11 of the 13 cases endometrial sarcoma was identified.
3. Tissues heterotopic to the uterus, such as striated muscle and cartilage, frequently appeared to originate in nests of endometrial sarcoma.

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PRIMARY OSTEOGENIC SARCOMA FOLLOWING X-IRRADIATION. Joe M. Blumberg and Robert Hufner (by invitation), Walter Reed Army Hospital, Washington, D.C.

Abstract. A group of 250 malignant tumors of the testis were treated surgically and by irradiation from 1940 through 1946. Four of these patients developed primary sarcomas in the irradiated sites. The first 2, previously treated at Walter Reed Army Hospital, have already been reported. This paper concerns the third of these cases to be necropsied. All had seminomas.

A white male, 27 years old, had a simple orchiectomy for seminoma of the right testis in November, 1942. From November 28, 1942, to February 12, 1943, he received 3,600 r. to each of six ports—pelvic, lumbar, and right and left lower dorsal. In recalculating the dosage to reach the para-aortic lymph nodes, an additional 4,000 r. was given to the right lower dorsal and right lumbar. He remained asymptomatic until March, 1951, when right upper quadrant pain and dyspnea resulted in hospitalization. A mass had developed in the border of the irradiated area which had received 7,600 r. This involved the right 10th rib posteriorly. Because the biopsy showed an anaplastic tumor, the patient was given another 7,000 r. intermittently to the tumor mass, both tangentially and directly, from May 1, 1951, through September 26, 1951. He had a rapidly progressive course attended by paralysis and atrophy of the leg muscles and died in November, 1951. Necropsy revealed an osteogenic sarcoma involving the entire right hemithorax and lumbar region. No evidence of seminoma was found. Radiation myelitis was present, having been produced by recent large doses of irradiation. This case, like the others, demonstrates that in treating one neoplasm of low grade malignancy, another malignant tumor may be induced. Therefore, the dangers involved must be considered when the method of

therapy is being selected. This case illustrates four complications of therapy: (a) primary irradiation osteogenic sarcoma, (b) irradiation myelitis, (c) irradiation ulcer of the pylorus, and (d) irradiation skin ulceration and necrosis of the skin.

The exact origin of this induced sarcoma is not known but an early x-ray examination showed a lesion involving the 10th rib. At necropsy, however, changes were noted that led to the possibility of its being muscular in origin with extension to bone, or of its having developed in both sites primarily. In Auerbach's case, which is similar in all respects, the origin was thought to be extra-skeletal.

THE HEMAGGLUTINATION TEST IN PULMONARY TUBERCULOSIS. Robert Cruickshank (by invitation) and Betty T. Wicks (by invitation), St. Mary's Hospital Medical School, London, England, and Western Reserve University Medical School, Cleveland, Ohio.

Abstract. An evaluation of the hemagglutination test of Middlebrook and Dubos in its relationship to pulmonary tuberculosis has been made. Modifications of the original technic have been used in an attempt to increase the specificity and sensitivity of the test. The results with sera for some 250 patients show a positive correlation with active pulmonary tuberculosis and indicate some relationship between the degree of positivity of the reaction and the activity and duration of the infection.

NECROTIZING GRANULOMATOSIS WITH ANGIITIS AND THE ATYPICAL PNEUMONITIDES. Robert Fienberg, Harvard Medical School and Cushing Veterans Administration Hospital, Boston and Framingham, Mass.

Abstract. In the first case, a discrete mass appeared in the lower lobe of the left lung without any sign of involvement of other organs. After the removal of the lower lobe containing the mass in which necrotizing granulomatosis with angiitis was found, the patient died of renal failure. Necropsy revealed discrete nodules in both lungs similar to those found at operation except for supervening acute necrosis. Generalized necrosis of the spleen, focal granulomatous thrombotic glomerulonephritis, and focal necrotizing granulomas in the lymph nodes, liver, stomach, small and large intestine, bladder, and skin also were present. Some of the arteries possessed lesions similar to those found in periarteritis nodosa. In the second case, death again was due to renal failure and the lungs, spleen, and kidneys contained lesions similar to those found in the same organs of the first case. The lesions in the lungs of these 2 cases, which contained fibroblastic proliferation, collagen deposition, an infiltrate of eosinophils, lymphocytes, plasma cells and histiocytes, abundant lipoid deposits, foci of necrosis with palisaded cells, angiitis with degeneration of the elastica, ulcerated bronchioles, and occasionally cavities, stimulated a study of 5 cases of discrete masses in the lungs of unknown cause removed at operation. In none of these 5 cases was there any evidence of involvement of other organs. In one, histologic details similar to those found in the lungs of the first 2 cases were discovered. In the other 4, the evidence was suggestive that a less intense but similar process was present. Bacteriologic studies revealed no specific pathogenic agent in either the post-mortem or the surgical specimens. In some of the cases, a history of sulfonamide therapy was obtained and in the first case, crystals resembling sulfonamide crystals were found in the renal tubules. It is thought that the lesions in all these cases represent an allergic reaction related to, but not identical with, periarteritis nodosa. Among other details, the peculiar nephritis and the vascular changes support this idea. The sulfonamides were probably the specific pathogenic agents in some of the cases. Finally, this study lends credence to the possibility that the lesions of the lungs described under various designations including atypical pneumonitis, Loeffler's syndrome, allergic granulomatosis, giant cell granuloma, periarteritis nodosa with granuloma of unknown etiology, rhinogenous granuloma (Wegener), pulmonary fibrosis, primary reticulo-endothelial granuloma (atypical Letterer-Siwe's disease), chronic

pneumonitis of cholesterol type, and eosinophilic xanthomatous granuloma belong to the same category.

THE MECHANISM OF ACTION OF CORTISONE ON EXPERIMENTAL SERUM SICKNESS. Frederick G. Germuth, Jr. (by invitation), Washington University Medical School, St. Louis, Mo.

Abstract. Experimental serum sickness was produced in rabbits by a single intravenous injection of crystalline bovine plasma albumin administered at a dosage of 0.25 gm. per kg. of body weight. When necropsied 12 days later, 24 per cent showed periarteritis nodosa; 71 per cent, well marked endocarditis; 40 per cent, focal interstitial myocarditis; 52 per cent, subendothelial infiltrations; and 80 per cent, acute glomerulitis. Of particular interest was an alteration found in 74 per cent of the spleens and 50 per cent of the lymph nodes examined. This consisted of an extensive infiltration of epithelioid cells and giant cells in or about the periphery of the malpighian bodies of the spleen and in the cortex of the lymph nodes. Similar lesions have been described in human cases of periarteritis nodosa.

The administration of cortisone prevented the development of the splenic lesions and all other lesions encountered in the controls. Serum antigen levels were determined at varying intervals after the injection of antigen by the quantitative precipitin method of Heidelberger and Kendall using a calibrated pooled antiserum. Cortisone had no effect on the rate of clearance of antigen from the serum and presumably no effect on antibody formation under the experimental conditions employed. It is concluded that the beneficial effect of cortisone on experimental serum sickness is not dependent on the suppression of antibody synthesis as is the active Arthus reaction. Cortisone may act by suppressing the injurious effects of antibody-antigen combination in the tissues.

THE MYOEPITHELIAL CELL IN THE NORMAL BREAST. J. Gershon-Cohen (by invitation) and Helen Ingleby, Jewish Hospital, Philadelphia, Pa.

Abstract. The purpose of this communication is to show the myoepithelial cells as they occur normally in the adolescent, non-pregnant, and pregnant human female breast, and to trace their development, especially in pregnancy.

EFFECT OF REPEATED X-RADIATION OF THE WHOLE BODY ON THE DEVELOPMENT OF TUMORS IN RATS FED *p*-DIMETHYL-AMINO-AZOBENZENE. Cornelia Hoch-Ligeti (by invitation), University of Virginia School of Medicine, Charlottesville, Va.

Abstract. The effects of x-radiation on the tumor process are two-fold: direct effects on cells involved in the tumor process and effects on systems connected with the defense mechanisms of the whole body. It seemed possible to distinguish between the direct and indirect effect of radiation in experiments in which x-rays are used in combination with a carcinogen not acting on the skin. The influence of total body irradiation on the development of hepatic tumors, which occur with great regularity in rats fed *p*-dimethyl-amino-azobenzene (PDA), seemed suitable for this purpose. Since the biologic effect of ionizing radiation is independent of the wave length, the experiments were arranged so that by using either penetrating or soft x-rays the direct effect due to radiation of the liver and the indirect effect due to radiation of the skin could be varied independently.

In the first experiment, reported previously, both soft and hard radiation were kept approximately equal as calculated for the dorsal skin surface. The rats fed PDA were irradiated postero-anteriorly. During the 76 weeks of the experiment the rats treated with penetrating radiation received 400 r. on the back, 310 r. in the liver, and a volume dose (calculated for 250 gm.) of about 100,000 (gm. × r.). The corresponding doses for rats treated with soft radiation were 310 r. in the skin of the back, 10 r. in the liver, and a volume dose of about 24,000 (gm. × r.).

While all non-irradiated rats fed PDA developed hepatic tumors, the development of tumors was prevented or their rates of growth retarded in rats treated with soft radiation. The growth of hepatic tumors was slightly retarded with penetrating radiation. In order to decide whether the difference in the amount of direct radiation of the liver or the difference in the volume doses was responsible for the different effects, their radiation in a second series was calculated so that the volume doses were approximately identical. Since in the first experiment 310 r. of soft radiation to the skin had protected the liver from tumors, this amount of soft radiation was used again, and the hard radiation was adjusted accordingly. With the hard radiation the rats received 92 r. to the dorsal surface, 9 r. to the liver, and a volume dose of 23,000 (gm. × r.) during the 65 weeks of the experiment. In the control group fed PDA but not receiving irradiation 12/18 rats developed hepatic tumors. Only 7/33 rats fed PDA and irradiated with soft rays had hepatic tumors. Twenty-three of the 30 rats fed PDA and irradiated with penetrating radiation had hepatic tumors. In one animal a squamous epithelioma of the skin occurred.

The adrenal glands of soft-irradiated animals were significantly increased in weight in both experiments. The pituitary bodies were heavier in the soft-irradiated group, but the difference was statistically barely significant. The weight of the spleens was unchanged. Testicular degeneration was found both in soft- and hard-irradiated animals.

These experiments indicate that the protective effect of irradiation on the livers of rats fed PDA is related neither to the dose of direct radiation received by the liver, nor to the total volume dose, but that it is related to the irradiation received by the skin. The effect might be metabolic and mediated perhaps through the adrenal and pituitary glands.

ANOXIA AS A CAUSE OF ENDOCARDIAL FIBROELASTOSIS IN INFANCY. Frank R. Johnson (by invitation), Children's Memorial Hospital and Northwestern University Medical School, Chicago, Ill.

Abstract. A study has been made of 23 hearts with diffuse endocardial fibroelastosis. These hearts were divided, by means of their gross appearance, into 8 groups and the literature reviewed briefly on each of the groups. It was found that endocardial fibroelastosis always develops in hearts having associated malformations which could produce endocardial anoxia. These malformations usually involved (1) an anomaly of the coronary arteries, which led to the delivery of unoxygenated blood to the endocardium, (2) valvular atresia in which stagnation anoxia developed, (3) premature closure of the foramen ovale, which prevented the flow of oxygenated blood into the left atrium and ventricle during intra-uterine life.

On the basis of this evidence it is hypothesized that an important factor in the development of so-called congenital diffuse endocardial fibroelastosis is cardiac anoxia, just as it is in endocardial scarring in the adult and best exemplified in myocardial infarction due to obstruction of the coronary arteries. There may, of course, be other factors. No evidence was obtained, however, that the lesion was due to intra-uterine infection or was a true congenital anomaly.

INTERSTITIAL PNEUMONIA AND FOCAL MYOCARDITIS IN POLIOMYELITIS. Seymour S. Jurow (by invitation) and Vera B. Dolgopol, Willard Parker Hospital, New York, N.Y.

Abstract. The visceral lesions observed in poliomyelitis have attracted much attention in the past 10 years. Focal myocarditis has been observed by a number of authors. A high incidence of interstitial pneumonia was noted in one report, on the basis of a small number of cases.

In this study the necropsy material of the Willard Parker Hospital from 1929 through 1951 was reviewed for the incidence of interstitial pneumonia and acute focal myocarditis. The heart lesions up to 1948 had been reviewed by one of us in

a previous publication; the material for the subsequent 4 years was reviewed in this study, and the incidence of acute focal myocarditis was brought up to date from 1929 through 1951. In all, the lungs were examined in 121 cases, the hearts in 120 cases. Interstitial pneumonia was observed in some parts of the lungs in 31 cases (25.6 per cent) of acute poliomyelitis and constituted 50 per cent of cases of all forms of pneumonia in our material (62 cases). It was the only inflammatory lesion of the lungs in 18 cases; in others, one or more lobes showed interstitial pneumonia, while another form of pneumonia was present in some other part of the lungs. In some instances the presence of interstitial pneumonia could be attributed to aspiration of regurgitated gastric contents. There was no correlation between the character of the pneumonic process and the age or sex of the patient, or the clinical type of poliomyelitis.

Acute focal myocarditis was found in 13 of 28 recent cases (46.4 per cent). In the 120 hearts examined 29 examples of focal myocarditis were encountered (24.1 per cent). If, however, only cases with multiple sections of the myocardium are included from the older study (45 cases of poliomyelitis, 11 cases of myocarditis) the final figures will be: 73 cases of poliomyelitis and 24 cases of acute focal myocarditis, or an incidence of 32.9 per cent.

Interstitial pneumonia, in combination with acute focal myocarditis, was encountered in 4 cases; severe bronchopneumonia was seen in 3 cases, and minor inflammatory changes were found in the lungs in 8 cases; no inflammatory changes were found in the lungs in 14 cases of acute focal myocarditis.

INTRAEPIDERMAL BASAL CELL EPITHELIOMA. Herbert Z. Lund, Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio.

Abstract. The usual basal cell epithelioma arises from the basal zone of the epidermis or from follicles. It is principally dermal in distribution and in its well differentiated forms imitates cutaneous appendages. In contrast there are lesions consisting of basal cells which tend to be intraepidermal and do not imitate adnexa. The nomenclature of this group is confusing. A review of a series of such tumors shows that they could be subdivided into several groups corresponding to descriptions in the literature: (1) seborrheic keratosis (basal cell papilloma); (2) intraepidermal basal cell epithelioma of Rose and Jadassohn; (3) intraepidermal basal and squamous cell epithelioma of Jadassohn; (4) reticular basal cell epithelioma. Common morphologic features were found. All are characterized by proliferation of small basal cells and a peculiar irregular branching overgrowth of dermal papillae which tends to separate and isolate the epithelium into a reticular pattern. Variations which have brought about the numerous subclassifications are in the degree of uniformity of basal cell proliferation whether focal or diffuse, the presence of simultaneous squamous cell proliferation, the amount of overgrowth, and the presence of cellular atypia and mitotic figures. Morphologically, all appear to be related and transitional appearances can be found. They are similar in behavior also. They do not metastasize, and penetration of the dermis is restricted to the papillary layer. The important differential diagnosis is from intraepidermal squamous cell or intermediary cell carcinomas which are tumors with more aggressive growth characteristics.

THE EFFECT OF BETA RAYS ON THE SKIN AS A FUNCTION OF THE ENERGY, INTENSITY, AND DURATION OF RADIATION. Alan R. Moritz and F. W. Henriques, Jr. (by invitation), Western Reserve University, Cleveland, Ohio, and Harvard Medical School, Boston, Mass.

Abstract. Exposure of the skin of young white pigs was made to beta radiation from plaques containing S^{35} (0.17 m.e.v.), Co^{60} (0.31 m.e.v.), Ce^{137} (0.55 m.e.v.), Yt^{91} (1.53 m.e.v.), and Sr-Yt^{90} (0.61 and 2.2 m.e.v.). The radiation emitted from each plaque was measured by means of a vanishing ion chamber and the percentile

reduction of the beta energy as it penetrated the tissue was computed for each isotope.

The critical factor in determining the occurrence and severity of injury is the amount of the surface dose that penetrates the living tissue. The characteristic epidermal alterations resulting from near-threshold exposures (1200 to 1700 r.e.b. at a depth of 0.9 mm. below the surface) was not recognized clinically or macroscopically and was manifested microscopically by a brief period of atrophy associated with occasional necrocytosis. Surface exposures sufficient to deliver a dosage of 2000 r.e.b. or more to a depth of 0.9 mm. were required for the production of chronic radiation dermatitis.

Chronic radiation dermatitis was manifested macroscopically by protracted exfoliation and recurrent ulceration. Microscopically, there were parakeratosis, persistent cytoplasmic and nuclear abnormalities in successive generations of epithelial cells, cicatrization, lymphocytic infiltration, and degenerative vascular changes in the dermis.

A Study of Vascular Reaction to Radiation: Rabbit Ear Chamber Technic. R. D. Moseley, Jr., C. C. Lushbaugh, and D. B. Hale (all by invitation), University of California and Los Alamos Scientific Laboratory, Los Alamos, N. Mex.

Abstract. The results of this study were recorded by kodachrome motion pictures. The first portion shows the method of Ebert of inserting lucite chambers in the external ears of rabbits. The second part shows typical changes in these revascularized chambers following lethal total body irradiation. In the typical experiment depicted, there was a vascularized chamber in each ear. With one ear shielded by lead, the other ear and the rest of the body of a rabbit were exposed to 1500 r. of 250 kv. x-radiation. Observations on both chambers were made microscopically at 30 minutes and at daily intervals for 7 days (the time of death) after irradiation. The capillaries in the shielded ear did not show the marked hyperemia and inability to react to neurochemical control which were seen in the irradiated ear. Leukocytes did not stick in the vessels protected from radiation and interstitial leukocytic exudation did not occur in this chamber although these changes were prominent in the irradiated chamber at 24 hours. The only changes seen in the protected chamber were sludging of the erythrocytes and paucity of leukocytes when the peripheral white blood cell count fell to leukopenic levels. It was concluded from the study of this rabbit and others that the effect of radiation on peripheral vessels is a direct one and that there is no evidence for the presence of a toxic circulating humoral agent causing these changes.

Splenomegaly and Kurloff Cell Hyperplasia in Guinea-Pigs Treated with Stilbestrol. Eli M. Nadel (by invitation), National Cancer Institute, Bethesda, Md.

Abstract. While studying the histopathology of estrogen-induced tumors in guinea-pigs and the interrelationships between stilbestrol and ascorbic acid metabolism, splenomegaly and a peculiar cell containing material which was stained positively by the periodic acid-leukofuchsin-sulfite (PAS) method were observed in the organs of 6-weeks-old guinea-pigs treated with stilbestrol for 14 days. The cell seen in tissue sections is identical morphologically and cytochemically with the Kurloff cell of the peripheral blood. The Kurloff cell was present in large numbers in the spleen where it comprised up to 65 per cent of the cell population in splenic impression prints from stilbestrol-treated animals. By the 14th day of stilbestrol treatment, up to 35 per cent of the leukocytes of the peripheral blood could be identified as Kurloff cells. Neither splenomegaly nor increased numbers of Kurloff cells were noted after subcutaneous implantation of testosterone, desoxycorticosterone, or methylcholanthrene pellets. The substance in the Kurloff cell is negative for iron and mucin, exhibits metachromasia with thionine, and does not lose its PAS-positivity when pre-

treated with amylase or hyaluronidase. Large numbers of Kurloff cells were present in the enlarged spleens of stilbestrol-treated guinea-pigs sacrificed 5 days after exposure to 550 r. of whole body radiation given in one dose during a period of 10 minutes. Additional histochemical and cytochemical observations have been made and are reported, and possible relations to hormone metabolism and radiation sickness protection are discussed.

NEURILEMMOMA OF THE STOMACH—AN INDIRECT CAUSE OF GASTRIC ULCERATION AND FULMINATING GASTRO-INTESTINAL HEMORRHAGE. REPORT OF TWO CASES. William R. Platt and James R. Eynon (by invitation), University of Pennsylvania Medical School, Philadelphia, Pa., and the West Jersey Hospital, Camden, N.J.

Abstract. Gastric neurilemmomas are peripheral visceral gliomas usually arising in the endodermal or mesodermal elements of stomach tissue and gradually growing in size until the overlying mucosa becomes ulcerated. The latter process is usually due to the neoplasm proliferating beneath the surface mucosa and not to direct infiltration of the lining mucous membrane cells. Since the ulceration and subsequent hemorrhage are functions of the tumor-produced mucosal pressure, it becomes obvious that bleeding will occur at different times in the life history of each neoplasm. For example, a small submucosal nodule growing into the stomach lumen usually produces ulceration of the mucous membrane much sooner than a larger subserous nodule growing outwardly into the peritoneal cavity. Bowel obstruction is rarely if ever produced; pain is minimal; and malignant change is rare. The tumor itself has a characteristic external and cut section appearance, and microscopically possesses the typical Verocay bodies observed in the type I variety. Gastric analysis, hematologic studies, and roentgenologic examination are correlative laboratory aids in the differential preoperative diagnosis. When gastro-intestinal bleeding of obscure origin is encountered, associated with minimal recurrent epigastric and/or upper abdominal pain, and when these symptoms do not respond to the usual conservative measures, neurilemmoma of the stomach should be considered as a possible cause.

METAPLASIA, HYPERPLASIA, AND NEOPLASIA IN THE UTERINE CERVIX DURING PREGNANCY. James W. Reagen and (by invitation) T. H. Redding and J. A. Merrill, Western Reserve University and University Hospitals of Cleveland, and Cleveland City Hospital, Cleveland, Ohio.

Abstract. The study is based on 226 pregnant patients chosen at random and subjected to biopsy, together with selected cases in which there were changes in the cervical squamous epithelium detected by cellular means. In the random cases the incidence of metaplasia was 38.88 per cent while 10.18 per cent showed a lesion of the endocervical mucosa classified as hyperplasia or atypical hyperplasia. An unusual degree of cellular proliferation was present in the epithelium of the portio vaginalis in 5.58 per cent of the cases. Only one neoplasm, an early invasive carcinoma, was seen in this group. The selected cases include examples of carcinoma *in situ* together with hyperplastic lesions, many of which show dysplasia or atypia. Evidence is presented to suggest the regression of certain changes in the epithelium when pregnancy is terminated.

SKELETAL DEVELOPMENT AND DEGENERATIVE JOINT DISEASE IN MICE TREATED WITH RADIOACTIVE IODINE (I^{131}). Martin Silberberg and Ruth Silberberg, Snodgras Laboratory, Hospital Division, City of St. Louis, and Washington University Medical School, St. Louis, Mo.

Abstract. In mice of strain Dba showing rapid skeletal development and aging, fat-enriched diets failed to promote skeletal aging and to increase the incidence of degenerative joint disease as they did in mice of the slowly aging strain C57 black. Since in microscopic sections the thyroid glands of Dba mice appeared more active

than those of C57 mice, differences in thyroid activity may have caused the above variations in skeletal response. The rôle of the thyroid gland in skeletal development and aging is, therefore, being reanalyzed.

In collaboration with Dr. Frank Dixon, Jr., I^{131} was injected intraperitoneally in order to produce hypo- or athyroidism. Dba mice weighing 10 to 14 gm. received 35 microcuries of I^{131} per gm. body weight, untreated animals of the same strain, age and sex serving as controls. One month after treatment, the growth zones showed decrease in the number of cartilage cells and retardation of the development of columnar into hypertrophic cartilage cells. Subsequently, the paucity of cells became accentuated, and the resorption of the hypertrophic cartilage cells was slowed down. At 6 months of age, the growth zones still had failed to narrow, and regressive changes in the epiphyseal cartilage were approximately of the same order as seen in 3 to 4 months-old controls; the resorption of cartilage and bone was further delayed, resulting in the presence of increased amounts of bone. Periosteal ossification remained little affected, although resorptive processes were likewise decreased.

The carcasses of 29 mice of strain C57 were obtained from Dr. Jacob Furth, Oak Ridge National Laboratories. These mice had received one dose (226 to 400 microcuries) of I^{131} at the ages of 1 to 3 months, and were sacrificed or died between 13 and 20 months of age (mean age 16.9 months). The growth zones of these mice were still wide and contained cartilage cells in columnar arrangement; advanced regressive changes were seen in the cartilage; the primary spongiosa had been resorbed, but epiphyseo-diaphyseal union had not occurred as it had in the controls. Thus, the appearance of the growth zones of 20-months-old test mice resembled that of 12-months-old controls. The thyroid deficiency manifested itself also in a delay of articular development and aging. Age changes were slight at best, and hardly noticeable before the age of 18 months, while severe articular age changes were seen in controls of even younger age. While in controls after the age of 15 months advanced osteo-arthritic lesions appeared in increasing numbers, none of the test animals showed severe osteo-arthritis. The lesions, if present at all, were focal in extent and slight in degree.

Experiments are in progress to determine how far the above results are due specifically to deficiency of thyroid hormone or are related to decreased caloric intake observed subsequent to thyroidectomy.

HISTOLOGY OF THE EXTERIORIZED GASTRIC MUCOSA OF THE RAT. C. E. Stevens (by invitation) and E. W. Toovey (by invitation), McGill University, Montreal, Canada.

Abstract. In a series of rats a flap of the greater curvature of the stomach was exteriorized on the anterior abdominal wall of the animal. Two animals were sacrificed daily for the first week, at weekly intervals for 7 weeks, and at monthly intervals for 1 year after operation. The flaps were then prepared for histologic examination.

The flaps survived over the whole year's period despite considerable change in the mucosa and a replacement of the submucosal structures by a highly vascular granulation tissue. During the first few postoperative days there occurred a rapid and almost complete degeneration of the mucosa with a marked inflammatory reaction, although usually one or two small areas at the edges of the flap retained the normal structure. An extremely rapid regeneration of the epithelium then ensued which mainly involved the deeper part of the mucosa as the surface was still infected. Regeneration took place from the remnants of the normal mucosa still present at the edges, and also started in the central region of the flap where all but a few of the deeper lying cells must have been destroyed. The newly formed cells were somewhat undifferentiated in appearance and contained a few apical granules of mucus, as shown by Southgate's mucicarmine and periodic acid-fuchsin, sulfurous acid stains. At 3 to 4 weeks the new cells were arranged like the bases of glands and were found at points across the whole surface of the flap. At 3 months the glands were developed

extensively so that the mucosa appeared adenoma-like. The deeper parts were composed of tortuous branching glands with occasional cysts of varying size. The cells were more differentiated than before and contained larger amounts of mucus. The cells of some parts of the glandular epithelium resembled mucous neck cells in that the mucus was distributed evenly throughout the cytoplasm. In other regions the mucus was concentrated in the apex of the cell as a theca, thus resembling the surface mucous cells. The surface of the flaps regenerated later than the glands and were mainly irregular in arrangement. This was presumably due to their exposure to mechanical trauma and frequent infection. Parts of the regenerated surface were composed of surface mucous cells. Small areas could still be found at the edges of some of the flaps which contained all the cells of the normal gastric mucosa which had survived since operation. A similar histologic picture was seen in all flaps examined up to 1 year after operation, with a great increase in the size of the flap due to the continued proliferation of the mucosa.

SOME EFFECTS OF CONCENTRATED AQUEOUS EXTRACTS OF MALIGNANT TUMORS ON CANCER PATIENTS. Emil Weiss, Peoples Hospital, Chicago, Ill.

Abstract. The extracts were prepared from a mixture of ground tumors which showed a high degree of malignancy grossly and microscopically, and a strong color reduction of dyes, described previously by me as characteristic for all malignant tumors. As a preservative, a solution of penicillin containing 200,000 units per 10 cc. of water was used. To 25 cc. of this solution 1 to 2 gm. of the ground tumor mixture was added and the suspension shaken vigorously for 10 to 15 minutes. The tissue extract was then filtered through several layers of linen. The extract was tested for sterility and on animals for toxicity. Every patient was further tested intracutaneously for sensitivity to penicillin. Two cc. of the extract were administered intramuscularly once a week for 6 consecutive weeks. Five hours after the injection of the extract, every patient reacted with a chill, lasting ½ to 2 hours, followed by a brief rise of temperature. The patients after a few injections showed a marked increase of appetite, felt stronger, and increased slightly in weight. A number of patients showed remarkable changes in the accessible regional lymph nodes. After a few injections the lymph nodes would diminish in size and become much harder. In some instances the shrinkage was preceded by necrosis and drainage of the liquefied material. Smears were prepared from the discharges and stained with Wright's or Giemsa's stain. The eosinophilic granulocytes frequently showed a definite increase. The epithelial cells differed greatly in size, shape and intensity of staining.

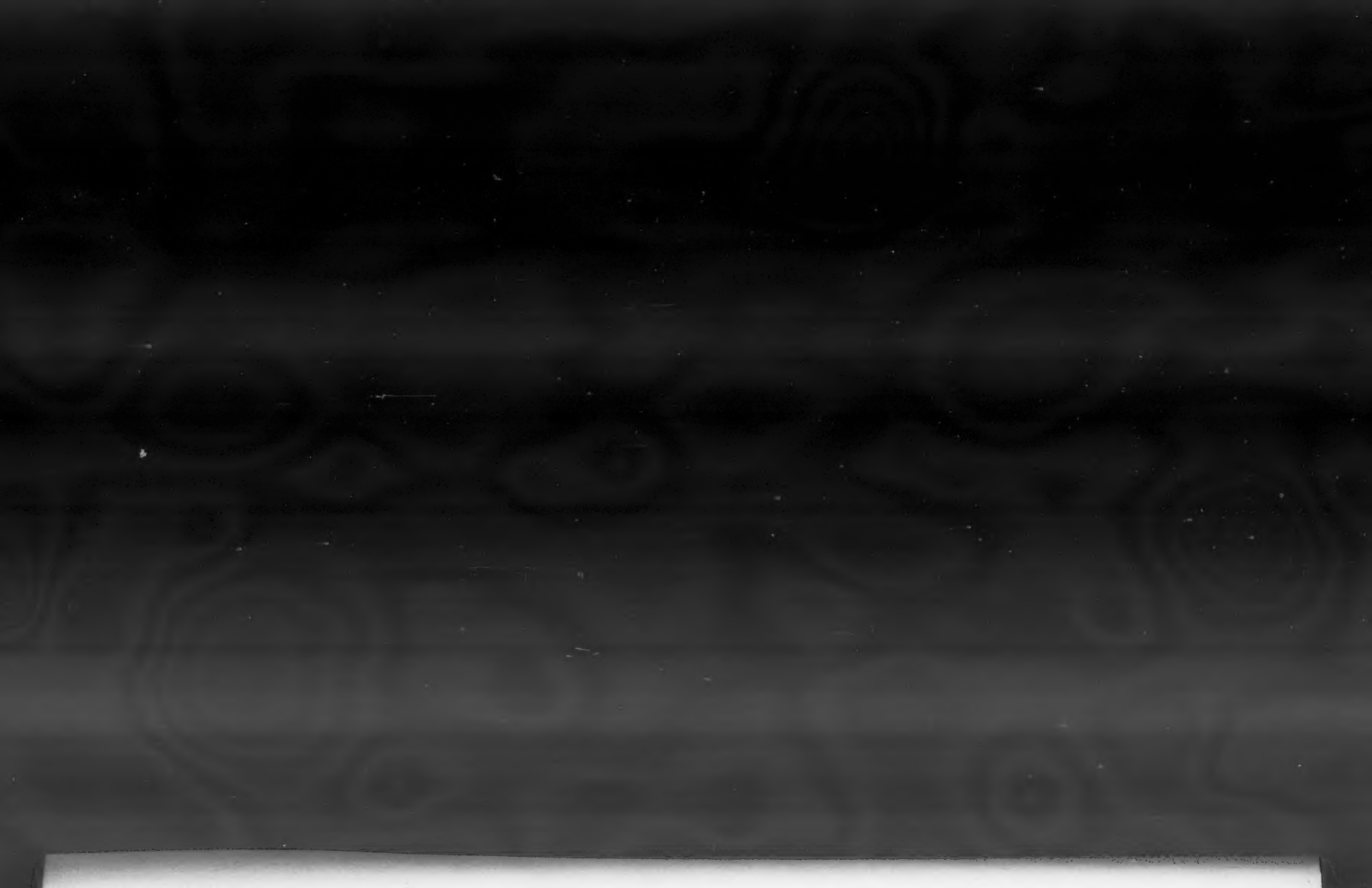
PRIMARY SYSTEMIC AMYLOIDOSIS (REPORT OF A CASE ASSOCIATED WITH BONE MARROW PLASMACYTOSIS, HYPERGLOBULINEMIA, AND PEMPHIGUS VULGARIS). Barney Worken (by invitation) and Julius Wolf (by invitation), Veterans Administration Hospital, Bronx, N.Y.

Abstract. This 55-year-old white male's illness began with a bullous eruption of the hands and feet. He had been treated 3 weeks previously with sulfonamides for "food poisoning." Two months later in another hospital there was noted a 3 plus albuminuria. Blood studies revealed 44 per cent lymphocytes, total serum protein of 8.53; serum albumin, 2.76; and serum globulin 4.68 gm. per 100 cc. (A/G ratio 0.6). In a sternal marrow smear about 50 per cent of the cell elements were identified as "myeloma" cells. A diagnosis of multiple myeloma and epidermolysis bullosa was made. Repeated examinations confirmed the relative and absolute lymphocytosis in the peripheral blood (45 to 50 per cent), increased serum globulin (4.1 to 5.0), and reduced serum albumin (2.6 to 2.8). Repeated tests for Bence Jones protein were negative. Several x-ray surveys of the skeletal system revealed no lytic areas or osteoporosis. A Congo red test was done and 75 per cent of the dye was retained in the serum at the end of 1 hour. Nine months after the onset the patient developed a myocardial infarct, verified by electrocardiogram, and died 6 months later.

The immediate cause of death was an embolus occluding the right internal carotid artery with widespread encephalomalacia of the right cerebral hemisphere. Microscopic examination revealed systemic amyloidosis with blood vessels and mesodermal structures selectively infiltrated, and the parenchyma of the liver, spleen, and adrenal glands spared entirely. The mitral and tricuspid valves were infiltrated with masses of amyloid, which produced a deformation seen grossly. Amyloid was present throughout the endocardium and focally in the myocardium. The coronary arterioles were converted into thickened tubes of amyloid, the lumina being narrowed extremely and often nearly obliterated. Amyloid deposition produced similar changes in the arterioles, medium-sized arteries, and veins of the skin, thyroid gland, lungs, liver, gallbladder, esophagus, gastro-intestinal tract, bladder, prostate, testes, pituitary body, and bone marrow. The vessels of the brain were spared. There was also amyloid infiltration of the tracheobronchial lymph nodes, skeletal muscle, bronchial musculature, and muscle layers of the esophagus and gastro-intestinal tract. Sections of the lumbar and thoracic vertebrae, ilium, ribs, and sternum revealed normal cortex and trabeculae, moderate hyperplasia of normal marrow elements, and a few plasma cells in the sternal and vertebral marrow. The diagnosis of pemphigus vulgaris was established from the post-mortem sections of the skin lesions.

INCIPIENT ADENOCARCINOMA OF THE STOMACH IN PEPTIC ULCER. REPORT OF TWO CASES. Raymond Yesner, Yale Medical School, and Veterans Administration Hospital, Newington, Conn.

Abstract. Of the last 20 carcinomas of the stomach removed surgically at Newington Veterans Administration Hospital, 2 were incipient adenocarcinomas arising in the wall of a peptic ulcer.

Case 1. A 55-year-old diabetic man with a 2-months history of weight loss, anorexia, and low back pain was found by x-ray to have a prepyloric ulcer with a meniscus carcinoma. A partial gastrectomy had been performed in May, 1948, and a figure-8-shaped ulcer was found at the pylorus with the intervening bridge exactly at the lesser curvature. Each oval half was shallow, 1 to 1.5 cm. in diameter, with a clean, white, fibrous base. On the anterior mucosal surface near the greater curvature, 2 cm. proximal to the pylorus, there was a 1 cm. polyp covered by normal-appearing mucosa. Microscopic sections showed a typical chronic peptic ulcer and a benign polyp. The patient was discharged in June. Because of poor appetite, no weight gain, and increasing weakness, he was readmitted in August, 1948. A sharply defined nodule in the left lower pulmonary lobe was found by x-ray. The gastric ulcer was therefore re-examined and multiple sections taken. In one small area an early adenocarcinoma was found at the gastric edge extending for a short distance into the submucosa. Lymph nodes were negative. Because of the possibility of a solitary metastasis, the left lower lobe was removed in April, 1949. A round, firm, 4 by 3.5 by 3.5 cm., solid, well circumscribed mass was found to be an anaplastic squamous cell carcinoma of the lung. Four years after gastrectomy and 3 years after lobectomy the patient is alive and well.

Case 2. A 67-year-old man was admitted in July, 1951. He had had symptoms of peptic ulcer for 8 years. In 1948 he was found by x-ray to have duodenal ulcer. On the present admission, narrowing of the pyloric antrum and a large ulcer on the lesser curvature were found, associated with high gastric acidity and partial obstruction. A partial gastrectomy was performed and two "kissing" ulcers were found in the prepyloric area, straddling the lesser curvature. They measured 0.5 to 1 cm. in diameter, had fibrotic floors and appeared to be typical peptic ulcers. Microscopic sections of one ulcer confirmed this impression. However, in one area of the wall of the other ulcer there was an early adenocarcinoma ***in situ***, composed of large, bizarre, disorganized cells. No invasion of the submucosa had yet occurred. Nine months after gastrectomy the patient is alive and well.